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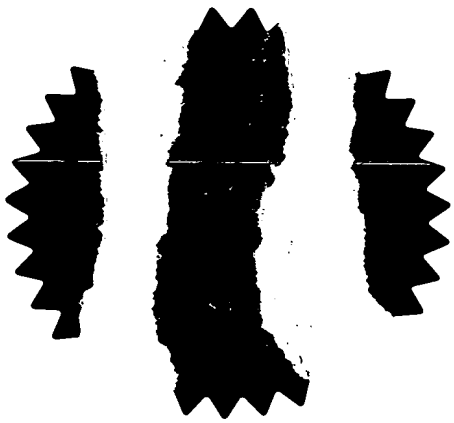

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MODULATORS

5. Name of your agent *(if you have one)*

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Modulators

Field of the invention

The present invention relates to the use of modulators of presenilin or presenilin-dependent gamma-secretase activity.

Background of the Invention

The etiological basis of Alzheimer's disease (AD) is not yet clear, however, a major portion of AD can be attributed to genetic factors. Familial Alzheimer's disease (FAD) is genetically heterogeneous and can be categorised according to age-of-onset using 60 years as a cut-off. The early-onset FAD genes include the amyloid ß-protein precursor (APP) gene on chromosome 21, the presenilin 1 (PS1) gene on chromosome 14, and the presenilin 2 (PS2) gene on chromosome 1. Approximately 50 % of early-onset FAD is accounted for by defects in these genes, with the majority occurring in the PS1 gene.

Presenilins have been implicated in Notch signalling and more particularly in the proteolysis of Notch to release its intracellular domain to the nucleus. Moreover gamma-secretases have been reported as also affecting this step in the Notch signalling pathway (DeStrooper). This is reviewed in Selkoe. Notch signal transduction also plays a critical role in cell fate determination in vertebrate and invertebrate tissues. Notch is expressed at many stages of Drosophila embryonic and larval development and in many different cells implying a wide range of functions including an important role in neurogenesis and in the differentiation of mesodermal and endodermal cells. Recent investigations have therefore concentrated on antagonists of presenilin in order to treat Alzheimer's diseases and other neural-associated diseases, and for altering the fate of a cell (see for example WO01/03743 and Hadland *et al.*).

During maturation in the thymus, T cells acquire the ability to distinguish self-antigens from those that are non-self, a process termed "self tolerance". Tolerance to a non-self antigen, however, may be induced by immunisation under specific conditions with a peptide fragment comprising that antigen. In autoimmune diseases such as multiple

sclerosis, rheumatoid arthritis or diabetes, there is a failure of the proper regulation of tolerance. Improved treatment methods for re-establishing tolerance are desirable for autoimmune diseases. Similarly in allergic conditions and for transplantation of an organ or tissue from a donor individual, induction of tolerance to particular foreign antigens or profiles of foreign antigens is desirable.

The expression on the cell surface of normal adult cells of the peripheral immune system of Notch and its ligands, Delta and Serrate, suggests a role for these proteins in T cell acquired immunocompetence (Hoyne et al. (2000) Int. Immunol, **12**:177-185). T cells express Notch mRNA constitutively. Delta expression is limited to only a subset of T cells in the peripheral lymphoid tissues. Serrate expression is restricted to a subset of antigen presenting cells (APCs). These observations reinforce the view that the Notch receptor ligand family continues to regulate cell fate decisions in the immune system beyond embryonic development (Ellisen) with Notch signalling playing a central role in the induction of peripheral unresponsiveness (tolerance or anergy), linked suppression and infectious tolerance.

Linked suppression occurs when an intact antigenic molecule is used for challenge immunisation and is characterised by cells being tolerised against, not only the target antigen, but also other, non-target regions of the antigenic molecule (Hoyne et al. (2000)). Infectious tolerance is a process whereby it is possible to generate a class of regulatory T cells which are able to transmit antigen-specific tolerance to other neighbouring T cells (Qin and WO98/20142). The functional activity of these cells can be mimicked by over-expression of a Notch ligand protein on their cell surfaces or on the surface of antigen presenting cells. In particular, regulatory T cells can be generated by over-expression of a member of the Delta or Serrate family of Notch ligand proteins. Delta or Serrate induced T cells specific to one antigenic epitope are also able to transfer tolerance to T cells recognising other epitopes on the same or related antigens, a phenomenon termed "epitope spreading".

Thus, as described in WO 98/20142, WO 00/36089 and WO 01/35990, manipulation of the Notch signalling pathway can be used in immunotherapy and in the prevention and/or treatment of T cell mediated diseases. In particular, allergy, autoimmunity, graft rejection, tumour induced aberrations to the T cell system and infectious diseases caused,

for example, by Plasmodium species, Microfilariae, Helminths, Mycobacteria, HIV, Cytomegalovirus, Pseudomonas, Toxoplasma, Echinococcus, Haemophilus influenza type B, measles, Hepatitis C or Toxicara, may be targeted. Notch ligand expression also plays a role in cancer. Indeed, upregulated Notch ligand expression has been observed in some tumour cells. These tumour cells are capable of rendering T cells unresponsive to restimulation with a specific antigen, thus providing a possible explanation of how tumour cells prevent normal T cell responses. Downregulation of Notch signalling *in vivo* in T cells may be used to prevent tumour cells from inducing immunotolerance in those T cells that recognise tumour-specific antigens. In turn, this allows the T cells to mount an immune response against the tumour cells (WO00/35990).

However, there remains a need in the art for the provision of further diagnostic or therapeutic compositions useful in the detection, prevention and treatment of T cell mediated diseases or disorders. The present invention addresses this problem.

Statements of the Invention

According to one aspect of the present invention, there is provided the use of a modulator of presenilin or presenilin-dependent gamma-secretase activity for the manufacture of a medicament for use in immunotherapy.

Put another way the present invention provides a method of immunotherapy comprising administering to an individual in need of the same an effective amount of a modulator of presenilin or presenilin-dependent gamma-secretase activity.

In a preferred embodiment the inventioon relates to use of a modulator of presenilin activity.

By "immunotherapy" we include the diagnosis, prevention or treatment of diseases, infections or conditions affected by the immune system.

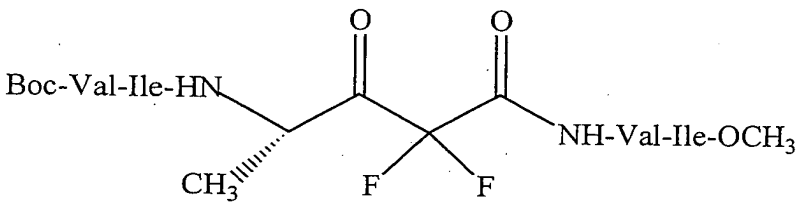
In one embodiment, the modulator of presenilin or presenilin-dependent gamma-secretase activity is used in combination with a modulator of the Notch signalling pathway.

In one embodiment, immunotherapy will involve the control of T cell activity including the treatment of a T cell mediated disease or infection, such as a T cell mediated disease or infection caused by any one or more of allergy, autoimmunity, graft rejection, tumour induced aberrations to the T cell and infectious diseases.

Examples of presenilin proteins which may be modulated in the present invention include Presenilin-1 (PS1) and Presenilin-2 (PS2).

The presenilin or presenilin-dependent gamma-secretase modulator will preferably be selected from polypeptides and fragments thereof, linear peptides, cyclic peptides, and nucleic acids which encode therefor, synthetic and natural compounds including low molecular weight organic or inorganic compounds and antibodies. The modulator may be an agonist or an antagonist of presenilin or presenilin-dependent gamma-secretase, optionally in combination with an agent capable of respectively up-regulating or down-regulating the Notch signalling pathway respectively.

Examples of agonists of presenilin which may be used in the present invention include Nicastrin or ALG-3 a nucleic acid sequence which encodes therefor. An example of an antagonist of presenilin which may be used in the present invention is 26S proteasome or a nucleic acid sequence which encodes therefor. Synthetic inhibitors include, for example, the difluoro ketone inhibitor described in Citron et al., and Wolfe et al. having the formula:



the inhibitors described in Sinha and Liederburg (2-Naphthoyl-VF-CHO, N-(2-Naphthoyl)-Val-phenylalaninal and N-Benzyloxycarbonyl-Leu-phenylalaninal Z-LF-CHO); the inhibitors described in Esler et al.; the inhibitors described in Figueiredo-Pereira et al., (N-Benzyloxycarbonyl-Leu-leucinal Z-LL-CHO); the inhibitors described in Higaki et al., (N-*trans*-3,5-Dimethoxycinnamoyl)-Ile-leucinal t-3,5-DMC-IL-CHO); the inhibitors described in Murphy et al., (Boc-GVV-CHO N-*tert*-Butyloxycarbonyl-Gly-Val-Valinal); and the inhibitors described in Riston et al., (1-(S)-*endo*-N-(1,3,3)-Trimethylbicyclo[2.2.1]hept-2-yl)-4-fluorophenyl Sulfonamide). Secretase activity may be inhibited by metalloproteases.

Agents capable of up-regulating expression the Notch signalling pathway and which may be used in the present invention include, but are not limited to, Notch ligands of the Serrate/Jagged and Delta families, Noggin, Chordin, Follistatin, Xnr3, FGF and derivatives, fragments, variants and homologues thereof, and immunosuppressive cytokines, or a combination thereof, and nucleic acid sequences which encode therefor. Agents capable of down-regulating the Notch signalling pathway and which may be used in the present invention include, but are not limited to, a Toll-like receptor, a cytokine, a bone morphogenetic protein (BMP), a BMP receptor or an activin or a nucleic acid sequence which encodes therefor.

In another aspect of the present invention there is provided a modulator of presenilin or presenilin-dependent gamma-secretase activity for use in immunotherapy.

In another aspect of the present invention there is provided a modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting a cell mediated disease, condition or infection.

In another aspect of the present invention there is provided a modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting linked suppression.

In another aspect of the present invention there is provided a modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting infectious tolerance.

In one embodiment, the modulator of presenilin or presenilin-dependent gamma-secretase will be used in combination with a modulator of the Notch signalling pathway as described above.

In another aspect of the present invention there is provided a method for producing a lymphocyte or antigen presenting cell (APC) having the ability to induce tolerance to an allergen or antigen which method comprises incubating a lymphocyte or APC obtained from a human or animal patient with (i) an agonist of presenilin or presenilin-dependent gamma-secretase and optionally an agent capable of up-regulating endogenous Notch or Notch ligand in the lymphocyte and/or APC and (ii) the allergen or antigen.

In one embodiment, the APC produced by the above method will be capable of inducing T cell tolerance. As such, there is preferably provided a method for producing *ex vivo* a T cell having tolerance to an allergen or antigen which method comprises incubating a T cell obtained from a human or animal patient with an antigen presenting cell (APC) in the presence of (i) an agonist of presenilin or presenilin-dependent gamma-secretase and optionally an agent capable of up-regulating expression of an endogenous Notch or Notch ligand in the APC and/or T cell and (ii) the allergen or antigen.

In another aspect of the present invention there is provided a method for producing a lymphocyte or APC having tolerance to an allergen or antigen which method comprises incubating a lymphocyte or APC obtained from a human or animal patient with a lymphocyte or APC produced by any one of the above described methods.

In one embodiment there is provided a method as described above for producing *ex vivo* a T cell having the ability to induce tolerance to an allergen or antigen which method comprises incubating a T cell obtained from a human or animal patient with a T cell produced by the method of any one of the above described methods.

In a further aspect of the present invention, there is provided the use of a lymphocyte or APC produced by any one of the methods of the invention in suppressing an immune response in a mammal to the allergen or antigen.

In another aspect of the present invention there is provided a method of treating a patient suffering from a disease characterised by inappropriate lymphocyte activity which method comprises administering to the patient a lymphocyte produced by the method of the invention.

In another aspect of the invention there is provided a method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating a T cell from a patient having said tumour cell present in their body;

(b) exposing the T cell to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell; and

(c) re-introducing the T cell into the patient;

wherein the T cell comprises a T cell receptor specific for a tumour antigen expressed by the tumour cell.

In another aspect of the present invention there is provided a method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating an antigen presenting cell (APC) from a tumour present in the body of a patient;

(b) exposing the APC to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell when the T cell is contacted with the APC; and

(c) re-introducing the APC into the patient.

In another aspect of the present invention there is provided a method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating a tumour cell from a tumour present in the body of a patient;

(b) exposing the tumour cell to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell when the T cell is contacted with the tumour cell; and

(c) re-introducing the tumour cell into the patient.

In one embodiment, the T cell used in the above methods is a tumour infiltrating lymphocyte (TIL).

In another aspect of the present invention there is provided a method of vaccinating a patient against a tumour which method comprises:

(a) administering a tumour antigen expressed by the tumour to the skin of the patient; and

(b) exposing the APC present in the skin of the patient to a modulator of or presenilin-dependent gamma-secretase activity which is capable of reducing or preventing expression, interaction or processing of Notch or a Notch ligand in a T cell.

In another aspect of the present invention, there is provided an assay method for modulators of presenilin or presenilin-dependent gamma-secretase activity comprising contacting a presenilin or presenilin-dependent gamma-secretase, respectively, in the presence of Notch and optionally a modulator of the Notch signalling pathway, with a candidate compound and determining if the compound affects the Notch signalling pathway.

In yet another aspect of the present invention there is provided an assay method for identifying substances that affect the interaction of a presenilin-interacting protein or presenilin-dependent gamma-secretase-interacting protein with a presenilin protein or presenilin-dependent gamma-secretase, respectively, comprising:

(a) providing a preparation containing: a presenilin protein or presenilin-dependent gamma-secretase; a presenilin-interacting protein or presenilin-dependent gamma-secretase-interacting protein, respectively; and a candidate substance; and

(b) detecting whether said candidate substance affects said interaction of said presenilin-interacting protein or presenilin-dependent gamma-secretase-interacting protein with said presenilin protein or presenilin-dependent gamma-secretase.

In one embodiment, the presenilin-interacting protein is Notch or a member of the Notch signalling pathway.

In a further aspect of the present invention, there is provided the use of a presenilin or presenilin-dependent gamma-secretase modulator identifiable using any one of the above assay methods in any one of the uses or methods of the invention.

In a yet further aspect of the present invention there is provided a kit comprising in one or more containers (a) a modulator of the Notch signalling pathway and (b) a modulator of or presenilin-dependent gamma-secretase activity.

Detailed Description

Various preferred features and embodiments of the present invention will now be described by way of non-limiting example and with reference to the accompanying drawings in which:

Figure 1 shows a schematic representation of the Notch signalling pathway;

Figure 2 shows a schematic representation of Notch proteins (Notch 1-4);

Figure 3 shows a schematic representation of the Notch intracellular domain; and

Figure 4 is a graph showing the results of the effect MW167 on Notch signalling in C2C12 cells transfected with mHes1-Luciferase.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J.

Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; *Current Protocols in Molecular Biology*, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, *DNA Isolation and Sequencing: Essential Techniques*, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, *In Situ Hybridization: Principles and Practice*; Oxford University Press; M. J. Gait (Editor), 1984, *Oligonucleotide Synthesis: A Practical Approach*, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, *Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA* Methods in Enzymology, Academic Press; and E. M. Shevach and W. Strober, 1992 and periodic suppliements, *Current Protocols in Immunology*, John Wiley & Sons, New York, NY. Each of these general texts is herein incorporated by reference.

For the avoidance of doubt, *Drosophila* and vertebrate names are used interchangeably throughout the description. Both (and where applicable, all) homologues are included within the scope of the invention.

Presenilin and the Notch signalling pathway

As used herein, the expression "Notch signalling" is synonymous with the expression "the Notch signalling pathway" and refers to any one or more of the upstream or downstream events that result in, or from, (and including) activation of the Notch receptor.

Preferably, by "Notch signalling" we refer to any event directly upstream or downstream of Notch receptor activation or inhibition including activation or inhibition of Notch/Notch ligand interactions, upregulation or downregulation of Notch or Notch ligand expression or activity and activation or inhibition of Notch signalling transduction including, for example, proteolytic cleavage of Notch and upregulation or downregulation of the Ras-Jnk signalling pathway.

Put another way, by "Notch signalling" we refer to the Notch signalling pathway as a signal tranducing pathway comprising elements which interact, genetically and/or molecularly, with the Notch receptor protein. For example, elements which interact with the Notch protein on both a molecular and genetic basis are, by way of example only, Delta, Serrate and Deltex. Elements which interact with the Notch protein genetically are, by way of example only, Mastermind, Hairless, Su(H) and Presenilin.

In one aspect, Notch signalling includes signalling events taking place extracellularly or at the cell membrane. In a further aspect, it includes signalling events taking place intracellularly, for example within the cell cytoplasm or within the cell nucleus.

A very important component of the Notch signalling pathway is Notch receptor/Notch ligand interaction. Thus the Notch signalling may involve changes in expression, nature, amount or activity of Notch ligands or receptors or their resulting cleavage products. In addition, the Notch signalling may involve changes in expression, nature, amount or activity of Notch signalling pathway G-proteins or Notch signalling pathway enzymes such as proteases, kinases (e.g. serine/threonine kinases), phosphatases, ligases (e.g. ubiquitin ligases) or glycosyltransferases. Alternatively the signalling may involve changes in expression, nature, amount or activity of DNA binding elements such as transcription factors.

In a preferred form of the invention the signalling detected is specific signalling, meaning that the signal detected results substantially or at least predominantly from the Notch signalling pathway, and preferably from Notch/Notch ligand interaction, rather than any other significant interfering or competing cause. The Notch signalling pathway is described in more detail below.

Notch signalling directs binary cell fate decisions in the embryo. Notch was first described in *Drosophila* as a transmembrane protein that functions as a receptor for two different ligands, Delta and Serrate. Vertebrates express multiple Notch receptors and ligands. At least four Notch receptors (Notch-1, Notch-2, Notch-3 and Notch-4) have been identified to date in human cells.

Notch proteins are synthesized as single polypeptide precursors that undergo cleavage via a Furin-like convertase that yields two polypeptide chains that are further processed to form the mature receptor. The Notch receptor present in the plasma membrane comprises a heterodimer of two Notch proteolytic cleavage products, one comprising an C-terminal fragment consisting of a portion of the extracellular domain, the transmembrane domain and the intracellular domain, and the other comprising the majority of the extracellular domain. Activation of Notch signalling involves proteolytic cleavage of the extracellular domain, involving TNF convertase (TACE), and intramembranous cleavage by presenilin-dependent γ-secretase activity.

Notch receptors are inserted into the membrane as disulphide-linked heterodimeric molecules consisting of an extracellular domain containing up to 36 epidermal growth factor (EGF)-like repeats and a transmembrane subunit that contains the cytoplasmic domain. The cytoplasmic domain of Notch contains six ankyrin-like repeats, a polyglutamine stretch (OPA) and a PEST sequence. A further domain termed RAM23 lies proximal to the ankyrin repeats and, like the ankyrin-like repeats, is involved in binding to a transcription factor, known as Suppressor of Hairless [Su(H)] in *Drosophila* and CBF1 in vertebrates (Tamura). The Notch ligands also display multiple EGF-like repeats in their extracellular domains together with a cysteine-rich DSL (Delta-Serrate Lag2) domain that is characteristic of all Notch ligands (Artavanis-Tsakonas).

The Notch receptor is activated by binding of extracellular ligands, such as Delta (Delta 1, 3 or 4), Serrate (Serrate 1 or 2 or their homologues Jagged 1 and 2) and Scabrous, to the EGF-like repeats of Notch's extracellular domain. Delta may require cleavage for activation. It is cleaved by the ADAM disintegrin metalloprotease Kuzbanian at the cell surface, the cleavage event releasing a soluble and active extracellular fragment of Delta. An oncogenic variant of the human Notch-1 protein, also known as TAN-1, which has a truncated extracellular domain, is constitutively active and has been found to be involved in T cell lymphoblastic leukemias.

The cdc10/ankyrin intracellular-domain repeats mediate physical interaction with intracellular signal transduction proteins. Most notably, the cdc10/ankyrin repeats

interact with Suppressor of Hairless [Su(H)]. Su(H) is the *Drosophila* homologue of C-promoter binding factor-1 [CBF-1], a mammalian DNA binding protein involved in the Epstein-Barr virus-induced immortalization of B-cells. It has been demonstrated that, at least in cultured cells, Su(H) associates with the cdc10/ankyrin repeats in the cytoplasm. Upon interaction of the Notch receptor with its ligand Delta on adjacent cells Su(H) disassociates from the Notch intracellular domain, where it is replaced by Deltex, and translocates into the nucleus. Su(H) interacts with responsive elements found in the promoters of several genes and has been found to be a critical downstream protein in the Notch signalling pathway. Target genes of Su(H) and of Notch signalling in general are listed below. The involvement of Su(H) in transcription is thought to be modulated by Hairless.

The intracellular domain of Notch (NotchIC) also has a direct nuclear function (Lieber). Recent studies have indeed shown that Notch activation requires that the six cdc10/ankyrin repeats of the Notch intracellular domain reach the nucleus and participate in transcriptional activation. The site of proteolytic cleavage on the intracellular tail of Notch has been identified between gly1743 and val1744 (termed site 3, or S3) (Schroeter). It is thought that the proteolytic cleavage step that releases the NotchIC for nuclear entry is dependent on Presenilin activity.

The intracellular domain has been shown to accumulate in the nucleus where it forms a transcriptional regulator complex with other transcription factors such as the CSL family member CBF1 (suppressor of hairless, Su(H) in *Drosophila*, Lag-2 in *C. elegans*) (Schroeter; Struhl) and Mastermind (MAML1/2). The NotchIC-CBF1 complexes then activate target genes, such as the bHLH proteins HES (hairy-enhancer of split like) 1 and 5 (Weinmaster). This nuclear function of Notch has also been shown for the mammalian Notch homologue (Lu).

NotchIC processing occurs only in response to binding of Notch ligands Delta or Serrate/Jagged. The post-translational modification of the nascent Notch receptor in the Golgi (Munro; Ju) appears, at least in part, to control which of the two types of ligand it interacts with on a cell surface. The Notch receptor is modified on its

extracellular domain by Fringe, a glycosyl transferase enzyme that binds to the Notch/Lin motif. Fringe modifies Notch by adding *O*-linked fucose groups to the EGF-like repeats (Moloney; Bruckner). This modification by Fringe does not prevent ligand binding, but may influence ligand induced conformational changes in Notch. Furthermore, recent studies suggest that the action of Fringe modifies Notch to prevent it from interacting functionally with Serrate/Jagged ligands but allow it to preferentially interact with Delta (Panin; Hicks). Although *Drosophila* has a single Fringe gene, vertebrates are known to express multiple genes (Radical, Manic and Lunatic Fringes) (Irvine).

Thus, signal transduction from the Notch receptor can occur via different pathways (Figure 1). The better defined pathway involves proteolytic cleavage of the intracellular domain of Notch (NotchIC) that translocates to the nucleus and forms a transcriptional activator complex with the CSL family protein CBF1 (supressor of hairless, Su(H) in *Drosophila*, Lag-2 in *C. elegans*). NotchIC-CBF1 complexes then activate target genes, such as Deltex or the bHLH proteins HES (hairy-enhancer of split like) 1 and 5. Notch can also signal in a CBF1-independent manner that involves the cytoplasmic zinc finger containing protein Deltex (Figure 1). Unlike CBF1, Deltex does not move to the nucleus following Notch activation. Instead, it interacts with Grb2 and modulates the Ras-Jnk signalling pathway which, in turn, modulates transcription of target genes.

Presenilins are integral membrane proteins with seven to eight transmembrane domains and a hydrophilic loop located between the transmembrane domains 6 and 7. Two presenilin genes have so far been identified: PS1 and PS2. More than 60% of amino acid residues in the sequence of PS1 and PS2 are conserved. The two proteins share major structural similarities, tissue-specific alternative splicing patterns and predicted tertiary structure. The presenilin genes have been identified as major causal genes for early onset familial Alzheimer's disease (FAD). FAD mutations are found throughout the entire PS1 molecule. However, two intramembranous aspartates at residues 257 and 385 have been revealed to be critical to the proper functioning of the protein (Capell *et al*).

Non-human homologues of the PS1 and PS2 genes and proteins have now been identified, isolated and cloned. Amongst them are the murine homologue (PS1) of human PS1, a *C. elegans* member (SEL-12) and a *D. melangoster* member (DmPS) of the presenilin gene family. Each of these genes and proteins have been identified on the basis of their high degrees of homology to the PS1/PS2 genes. Modulators of any of these genes and proteins, or any others which are known or become available, are included in the scope of the present invention.

Modulators of Presenilin or Presenilin-dependent gamma-secretase

The term "modulate" as used herein in relation to presenilin or presenilin-dependent gamma-secretase refers to a change or alteration in the biological activity of presenilin. Thus, modulation of presenilin or presenilin-dependent gamma-secretase activity includes inhibition or down-regulation of Notch signalling, e.g. by compounds which block, at least to some extent, the normal biological activity of presenilin or presenilin-dependent gamma-secretase. Alternatively, the term "modulation" may refer to the activation or up-regulation of presenilin or presenilin-dependent gamma-secretase activity, e.g. by compounds which stimulate or upregulate, at least to some extent, the normal biological activity of presenilin or presenilin-dependent gamma-secretase.

In other words, modulators of presenilin or presenilin-dependent gamma-secretase include compounds capable of activating or inhibiting the expression and/or activity of presenilin or presenilin-dependent gamma-secretase.

Presenilin or Presenilin-dependent gamma-secretase activators

By a compound capable of activating presenilin or presenilin-dependent gamma-secretase, we refer to compounds capable of activating any one or more of the polypeptides or polynucleotides of the presenilin or presenilin-dependent gamma-secretase family, in particular PS1 and PS2, and any homologues, derivatives or variants thereof.

In one embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase activation will be a dominant negative version of a presenilin or presenilin-dependent gamma-secretase repressor, respectively. In an alternative embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase activation will be capable of inhibiting a presenilin or presenilin-dependent gamma-secretase repressor, respectively. In a further alternative embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase activation will be a positive activator of presenilin or presenilin-dependent gamma-secretase, respectively.

In a particular embodiment, the molecule will be capable of inducing or increasing presenilin or presenilin-dependent gamma-secretase expression. Such a molecule may be a nucleic acid sequence capable of inducing or increasing presenilin or presenilin-dependent gamma-secretase expression.

In one embodiment, the molecule will be capable of up-regulating expression of endogenous presenilin or presenilin-dependent gamma-secretase in target cells. In particular, the molecule may be an immunosuppressive cytokine capable of up-regulating the expression of endogenous presenilin or presenilin-dependent gamma-secretase in target cells, or a polynucleotide which encodes such a cytokine. Immunosuppressive cytokines include IL-4, IL-10, IL-13, TGF-β and FLT3 ligand.

Preferably, the molecule will be a polypeptide selected from polypeptides of the ALG family, in particular ALG-3, Nicastrin, Calsenilin, β-catenin or Bcl-X(L), or variants, derivatives or fragments thereof or a polynucleotide encoding such a polypeptide or a variant, derivative or fragment thereof.

ALG-3 is a mouse homologue of the Chromosome 1 familial Alzheimer's disease gene PS2. It codes for a truncated PS2 polypeptide (the 103 COOH-terminal PS2 amino-acids) that is capable of inhibiting the apoptotic role of PS2 (D'Adamino *et al*). It has indeed been found that PS2 is required for some forms of cell death in diverse cell types and that ALG-3 rescues mouse T hybridoma 3DO cells from T cell receptor-induced apoptosis by inhibiting Fas ligand induction and Fas signalling (Lacana *et al*). ALG-3 has also been shown to reduce protease activity and to

antagonise polymerase cleavage upon Fas triggering. The polynucleotide sequence of ALG-3 can be found at GenBank Accession Number U49111.

Nicastrin is a type 1 transmembrane glycoprotein which has a domain found in the aminopeptidase/transferrin receptor superfamily (Fagan *et al*). It acts as a key regulator for presenilin-mediated gamma-secretase cleavage of β-amyloid precursor protein by forming a functional complex with PS1 and PS2. It plays a central role in presenilin mediated processing of Notch (Gang *et al*). Suppression of Nicastrin expression in *C. elegans* embryos induces a subset of Notch phenotypes similar to those induced by simultaneous null mutations in both presenilin homologues of *C. elegans*. Thus, it is thought that Nicastrin and presenilins are functional components of a multimeric complex necessary for the intermembranous proteolysis of the Notch protein. The polynucleotide sequence of Nicastrin can be found at GenBank Accession Numbers NM_021607 (Mus musculus), AF240470 (Drosophila melanogaster) and AF240468 (Homo sapiens).

Bcl-X(L), an anti-apoptotic member of the Bcl-2 family, has been shown to interact with the carboxyl-terminal fragments of PS1 and PS2 (Passer *et al*). Furthermore, it has been demonstrated that Bcl-X(L) and PS2 partially co-localise to sites of the vesicular transport system. The polynucleotide sequence of Bcl-X(L) can be found at GenBank Accession Number NM_004050.

Calsenilin is a member of the recoverin family of neuronal calcium binding proteins that have been shown to interact with PS1 and PS2. Calsenilin has the ability to interact with the endogenous 25-kDa presenilin C-terminal fragment that is produced by regulated endoproteolytic cleavage. Thus, the expression of calsenilin can regulate levels of an active proteolytic product of presenilin (Choi *et al*). The polynucleotide sequence of calsenilin can be found at GenBank Accession Numbers NM_032462 (Rattus norvegicus), XM_015414 (Homo sapiens) and NM_019789 (Mus musculus).

β-catenin binds PS1 in an interaction thought to mechanistic in Alzheimer's disease. The cyclin-dependent kinase p35/cdk5 binds and phosphorylates β-catenin thus

regulating its interaction with PS1 (Kesavapany *et al*). The polynucleotide sequence of Xenopus laevis β-catenin can be found at GenBank Accession Number M77013.

In a preferred embodiment, the activator will be a constitutively active presenilin or presenilin-dependent gamma-secretase or a homologue, variant, derivative or fragment thereof or a polynucleotide encoding such a presenilin. Alternatively, the activator may be a molecular mimic of a constitutively active presenilin or presenilin-dependent gamma-secretase.

By polypeptides or polynucleotides for presenilin or presenilin-dependent gamma-secretase activation, we also include molecules activated or expressed as a result of presenilin or presenilin-dependent gamma-secretase activation and any compounds involved in the activation or expression of such molecules. Examples of such molecules are the Notch Intracellular Domain (NICD), the CSL family protein CBF1 (Su(H) in *Drosophila*, Lag-2 in *C. elegans*), bHLH proteins HES1 and HES5.

Activation of presenilin or presenilin-dependent gamma-secretase may also be achieved by repressing inhibitors of presenilin or presenilin-dependent gamma-secretase, respectively. As such, presenilin or presenilin-dependent gamma-secretase activators include molecules capable of repressing any presenilin or presenilin-dependent gamma-secretase inhibitors, respectively. Preferably, the molecule will be a polypeptide, or a polynucleotide encoding such a polypeptide, that decreases or interferes with the production or activity of compounds that are capable of producing a decrease in the expression or activity of presenilin or presenilin-dependent gamma-secretase. In a preferred embodiment, the molecules will be capable of repressing polypeptides such as 26S proteasome.

Presenilin or Presenilin-dependent gamma-secretase inhibitors

By a polypeptide capable of inhibiting presenilin or presenilin-dependent gamma-secretase, we mean a molecule capable of inhibiting any one or more of the polypeptides or polynucleotides of the presenilin or presenilin-dependent gamma-secretase family, in particular PS1 and PS2, and any homologues, derivatives or variants thereof.

In one embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase inhibition will be a dominant negative version of a presenilin or presenilin-dependent gamma-secretase activator, respectively. In an alternative embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase inhibition will be capable of inhibiting a presenilin or presenilin-dependent gamma-secretase activator, respectively. In a further alternative embodiment, the molecule for presenilin or presenilin-dependent gamma-secretase activation will be a direct repressor of presenilin or presenilin-dependent gamma-secretase, respectively.

In a particular embodiment, the molecule will be capable of reducing or preventing presenilin or presenilin-dependent gamma-secretase expression. Such a molecule may be a nucleic acid sequence capable of reducing or preventing presenilin or presenilin-dependent gamma-secretase expression.

In one embodiment, the molecule will be capable of down-regulating expression and/or activity of endogenous presenilin or presenilin-dependent gamma-secretase in target cells. Preferably, the molecule will be a polypeptide selected from polypeptides of the proteasome family, in particular 26S proteasome or Sel 10 and its mammalian homologues, or variants, derivatives or fragments thereof or a polynucleotide encoding such a polypeptide or a variant, derivative or fragment thereof.

26S proteasome is capable of degrading PS1 by causing endoproteolytic cleavage of the protein near residue 298 (Fraser *et al*).

In an another embodiment, the inhibitor will be a polypeptide, or a polynucleotide encoding such a polypeptide, that decreases or interferes with the production of compounds that are capable of producing an increase in the expression of presenilin or presenilin-dependent gamma-secretase.

Alternatively, the inhibitor is a polynucleotide, preferably an antisense construct derived from a sense nucleotide sequence encoding a polypeptide selected from presenilin or presenilin-dependent gamma-secretase and presenilin or presenilin-

dependent gamma-secretase activators such as ALG-3, Nicastrin, Calsenilin, β-catenin or Bcl-X(L), derivatives, fragments, variants and homologues thereof.

Inhibitors of presenilin further include compounds capable of repressing the expression or activity of molecules normally activated by the expression or activity of presenilin or presenilin-dependent gamma-secretase (e.g. CBF1, HES1 or HES5) and compounds the expression or activity of which is normally repressed by that of presenilin or presenilin-dependent gamma-secretase. Proteins for presenilin or presenilin-dependent gamma-secretase inhibition will also include variants of the above described activators of presenilin or presenilin-dependent gamma-secretase which have been modified in such a way as to block rather than activate or transduce presenilin or presenilin-dependent gamma-secretase. An example of such an inhibitor would be a presenilin protein or presenilin-dependent gamma-secretase modified in such a way that it binds to but does not cleave Notch.

Modulators of the Notch signalling pathway

In a preferred embodiment of the present invention, the modulator of presenilin or presenilin-dependent gamma-secretase is used in conjunction with a modulator of the Notch signalling pathway, i.e. a compound capable of up-regulating or down-regulating the Notch signalling pathway.

The term "modulate" as used herein refers to a change or alteration in the biological activity of the Notch signalling pathway or a target signalling pathway thereof. Thus, modulation of Notch signalling includes inhibition or down-regulation of Notch signalling, e.g. by compounds which block, at least to some extent, the normal biological activity of the Notch signalling pathway. Alternatively, the term "modulation" may refer to the activation or up-regulation of Notch signalling, e.g. by compounds which stimulate or upregulate, at least to some extent, the normal biological activity of the Notch signalling pathway.

Up-regulators of the Notch signalling pathway

Compounds capable of up-regulating the Notch signalling pathway are compounds capable of transducing or activating the Notch signalling pathway. By a polypeptide or polynucleotide which is for Notch signalling transduction we include a molecule which participates in signalling through Notch receptors including activation of Notch, the downstream events of the Notch signalling pathway, transcriptional regulation of downstream target genes and other non-transcriptional downstream events (e.g. post-translational modification of existing proteins). More particularly, the second sequence is a domain that allows activation of target genes of the Notch signalling pathway, or a polynucleotide sequence which codes therefor.

In other words, by modulating Notch signalling transduction we include:

a) activation of the Notch signalling pathway by (i) dominant negative or inhibitors of repressors and (ii) activators; and
b) blockade of the Notch signalling pathway by (i) dominant negative or inhibitors of activators and (ii) inhibitors.

Key targets for Notch-dependent transcriptional activation are genes of the *Enhancer of split* complex (E[spl]). Moreover these genes have been shown to be direct targets for binding by the Su(H) protein and to be transcriptionally activated in response to Notch signalling. By analogy with EBNA2, a viral coactivator protein that interacts with a mammalian Su(H) homologue CBF1 to convert it from a transcriptional repressor to a transcriptional activator, the Notch intracellular domain, perhaps in association with other proteins may combine with Su(H) to contribute an activation domain that allows Su(H) to activate the transcription of *E(spl)* as well as other target genes. It should also be noted that Su(H) is not required for all Notch-dependent decisions, indicating that Notch mediates some cell fate choices by associating with other DNA-binding transcription factors or by employing other mechanisms to transduce extracellular signals.

According to one aspect of the present invention the second sequence is the Notch polypeptide or polynucleotide or a fragment thereof which retains the signalling transduction ability of Notch or an analogue of Notch which has the signalling transduction ability of Notch. By Notch, we mean Notch-1, Notch-2, Notch-3, Notch-4 and any other Notch homologues or analogues. In a particularly preferred

embodiment the second amino acid sequence is the Notch intracellular domain (Notch IC) or a sub-fragment thereof.

As used herein the term "analogue of Notch" includes variants thereof which retain the signalling transduction ability of Notch. By "analogue" we include a protein which has Notch signalling transduction ability, but generally has a different evolutionary origin to Notch. Analogues of Notch include proteins from the Epstein Barr virus (EBV), such as EBNA2, BARF0 or LMP2A.

By a polypeptide or polynucleotide which is for Notch signalling activation we mean a molecule which is capable of activating Notch, the Notch signalling pathway or any one or more of the components of the Notch signalling pathway.

In one embodiment, the molecule for Notch signalling activation will be a dominant negative version of a Notch signalling repressor. In an alternative embodiment, the molecule for Notch signalling activation will be capable of inhibiting a Notch signalling repressor. In a further alternative embodiment, the molecule for Notch signalling activation will be a positive activator of Notch signalling.

In a particular embodiment, the molecule will be capable of inducing or increasing Notch or Notch ligand expression. Such a molecule may be a nucleic acid sequence capable of inducing or increasing Notch or Notch ligand expression.

In one embodiment, the molecule will be capable of up-regulating expression of the endogenous genes encoding Notch or Notch ligands in target cells. In particular, the molecule may be an immunosuppressive cytokine capable of up-regulating the expression of endogenous Notch or Notch ligands in target cells, or a polynucleotide which encodes such a cytokine. Immunosuppressive cytokines include IL-4, IL-10, IL-13, TGF-β and FLT3 ligand.

Preferably, the molecule will be a polypeptide selected from Noggin, Chordin, Follistatin, Xnr3, fibroblast growth factors and derivatives, fragments, variants and homologues thereof, or a polynucleotide encoding any one or more of the above.

In another embodiment, the molecule may be a Notch ligand, or a polynucleotide encoding a Notch ligand. Notch ligands of use in the present invention include endogenous Notch ligands which are typically capable of binding to a Notch receptor polypeptide present in the membrane of a variety of mammalian cells, for example hemapoietic stem cells.

Particular examples of mammalian Notch ligands identified to date, and of use in the present invention, include the Delta family, for example Delta (Genbank Accession No. AF003522 - *Homo sapiens*), Delta-3 (Genbank Accession No. AF084576 - *Rattus norvegicus*) and Delta-like 3 (*Mus musculus*), and the Serrate family, for example Serrate-1 and Serrate-2 (WO97/01571, WO96/27610 and WO92/19734), Jagged-1 and Jagged-2 (Genbank Accession No. AF029778 - *Homo sapiens*), and LAG-2. Homology between family members is extensive. For example, human Jagged-2 has 40.6% identity and 58.7% similarity to Serrate.

In a preferred embodiment, the activator will be a constitutively active Notch receptor or Notch intracellular domain, or a polynucleotide encoding such a receptor or intracellular domain.

In an alternative embodiment, the activator of Notch signalling will act downstream of the Notch receptor. Thus, for example, the activator of Notch signalling may be a constitutively active Deltex polypeptide or a polynucleotide encoding such a polypeptide. Other downstream components of the Notch signalling pathway of use in the present invention include Deltex-1, Deltex-2, Deltex-3, Suppressor of Deltex (SuDx), Numb and isoforms thereof, Numb associated Kinase (NAK), Notchless, Dishevelled (Dsh), emb5, Fringe genes (such as Radical, Lunatic and Manic), Fringe Connection PON, LNX, Disabled, Numblike, Nur77, NFkB2, Mirror, Warthog, Engrailed-1 and Engrailed-2, Lip-1 and homologues thereof, the polypeptides involved in the Ras/MAPK cascade modulated by Deltex, polypeptides involved in the proteolytic cleavage of Notch such as Presenilin and polypeptides involved in the transcriptional regulation of Notch target genes, preferably in a constitutively active form, and analogues, derivatives, variants and fragments thereof.

By polypeptides or polynucleotides for Notch signalling activation is also meant any

polypeptides expressed as a result of Notch activation and any polypeptides involved in the expression of such polypeptides, or polynucleotides encoding for such polypeptides.

Activation of Notch signalling may also be achieved by repressing inhibitors of the Notch signalling pathway. As such, polypeptides for Notch signalling activation will include molecules capable of repressing any Notch signalling inhibitors. Preferably the molecule will be a polypeptide, or a polynucleotide encoding such a polypeptide, that decreases or interferes with the production or activity of compounds that are capable of producing a decrease in the expression or activity of Notch, Notch ligands, or any downstream components of the Notch signalling pathway. In a preferred embodiment, the molecules will be capable of repressing polypeptides of the Toll-like receptor protein family, cytokines such as IL-12, IFN-γ, TNF-α, and growth factors such as the bone morphogenetic protein (BMP), BMP receptors and activins, derivatives, fragments, variants and homologues thereof.

Down-regulators of Notch signalling pathway

By a polypeptide or polynucleotide which is for Notch signalling inhibition, we mean a molecule which is capable of inhibiting Notch, the Notch signalling pathway or any one or more of the components of the Notch signalling pathway.

In one embodiment, the molecule for Notch signalling inhibition will be a dominant negative version of a compound capable of activating or transducing Notch signalling. In an alternative embodiment, the molecule for Notch signalling inhibition will be capable of repressing a compound capable of activating or transducing Notch signalling. In a further alternative embodiment, the molecule for Notch signalling inhibition will be an inhibitor of Notch signalling.

In a particular embodiment, the molecule will be capable of reducing or preventing Notch or Notch ligand expression. Such a molecule may be a nucleic acid sequence capable of reducing or preventing Notch or Notch ligand expression.

Preferably the nucleic acid sequence encodes a polypeptide selected from Toll-like receptor protein family, a cytokine such as IL-12, IFN-γ, TNF-α, or a growth factor such as a bone morphogenetic protein (BMP), a BMP receptor and activins. Preferably the agent is a polypeptide, or a polynucleotide encoding such a polypeptide, that decreases or interferes with the production of compounds that are capable of producing an increase in the expression of Notch ligand, such as Noggin, Chordin, Follistatin, Xnr3, fibroblast growth factors and derivatives, fragments, variants and homologues thereof.

Alternatively, the nucleic acid sequence is an antisense construct derived from a sense nucleotide sequence encoding a polypeptide selected from a Notch ligand and a polypeptide capable of up-regulating Notch ligand expression, such as Noggin, Chordin, Follistatin, Xnr3, fibroblast growth factors and derivatives, fragments, variants and homologues thereof.

In another preferred embodiment the inhibitor of Notch signalling is a molecule which is capable of modulating Notch-Notch ligand interactions. A molecule may be considered to modulate Notch-Notch ligand interactions if it is capable of inhibiting the interaction of Notch with its ligands, preferably to an extent sufficient to provide therapeutic efficacy.

In this embodiment the molecule may be a polypeptide, or a polynucleotide encoding such a polypeptide, selected from a Toll-like receptor, a cytokine such as IL-12, IFN-γ, TNF-α, or a growth factor such as a BMP, a BMP receptor and activins. Preferably the polypeptide decreases or interferes with the production of an agent that is capable of producing an increase in the expression of Notch ligand, such as Noggin, Chordin, Follistatin, Xnr3, fibroblast growth factors and derivatives, fragments, variants, homologues and analogs thereof.

Preferably when the inhibitor is a receptor or a nucleic acid sequence encoding a receptor, the receptor is activated. Thus, for example, when the agent is a nucleic acid sequence, the receptor is constitutively active when expressed.

Inhibitors of Notch signalling also include downstream inhibitors of the Notch signalling pathway (such as Dsh and Numb), compounds that prevent expression of Notch target genes or induce expression of genes repressed by the Notch signalling pathway and dominant negative versions of Notch signalling transducer molecules (such as of Notch IC and Deltex). Proteins for Notch signalling inhibition will also include variants of the wild-type components of the Notch signalling pathway which have been modified in such a way that their presence blocks rather than transduces the signalling pathway. An example of such a compound would be a Notch receptor which has been modified such that proteolytic cleavage of its intracellular domain is no longer possible.

Cells of use in the invention

Cells of use in the present invention may be tumour cells or cells of the immune system and will be capable of transducing the Notch signalling pathway.

Tumour cells expressing Notch ligand

The expression of Notch ligands in melanoma cell lines has been identified. Other tumour cells may also be tested for expression of Notch ligands using a variety of techniques known in the art such as detection of mRNA by RT-PCR or detection of the Notch ligand polypeptides by Western blotting. Suitable tumour cells to be tested include cells present in malignancies such as cancer of the breast, cervix, colon, rectum, endometrium, kidney, lung, ovary, pancreas, prostate gland, skin, stomach, bladder, CNS, oesophagus, head-or-neck, liver, testis, thymus or thyroid. Malignant blood cells, bone marrow cells, B-lymphocytes, T-lymphocytes, lymphocytic progenitors or myeloid cell progenitors may also be tested.

Tumour cells which express Notch ligand may be a tumour cells from a solid tumour or a non-solid tumour and may be a primary tumour cell or a disseminated metastatic (secondary) tumour cell. Non-solid tumours include myeloma; leukaemia (acute or chronic, lymphocytic or myelocytic) such as acute myeloblastic, acute promyelocytic, acute myelomonocytic, acute monocytic, erythroleukaemia; and lymphomas such as Hodgkin's, non-Hodgkin's and Burkitt's. Solid tumours include carcinoma, colon

carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, adenocarcinoma, melanoma, basal or squamous cell carcinoma, mesothelioma, adenocarcinoma, neuroblastoma, glioma, astrocytoma, medulloblastoma, retinoblastoma, sarcoma, osteosarcoma, rhabdomyosarcoma, fibrosarcoma, osteogenic sarcoma, hepatoma, and seminoma.

Antigen Presenting Cells

Antigen-presenting cells (APCs) for use in the present invention may be "professional" antigen presenting cells or may be another cell that may be induced to present antigen to T cells. Alternatively a APC precursor may be used which differentiates or is activated under the conditions of culture to produce an APC. An APC for use in the *ex vivo* methods of the invention is typically isolated from a tumour or peripheral blood found within the body of a patient. Preferably the APC or precursor is of human origin. However, where APCs are used in preliminary *in vitro* screening procedures to identify and test suitable nucleic acid sequences, APCs from any suitable source, such as a healthy patient, may be used.

APCs include dendritic cells (DCs) such as interdigitating DCs or follicular DCs, Langerhans cells, PBMCs, macrophages, B-lymphocytes, T-lymphocytes, or other cell types such as epithelial cells, fibroblasts or endothelial cells, activated or engineered by transfection to express a MHC molecule (Class I or II) on their surfaces. Precursors of APCs include $CD34^+$ cells, monocytes, fibroblasts and endothelial cells. The APCs or precursors may be modified by the culture conditions or may be genetically modified, for instance by transfection of one or more genes encoding proteins which play a role in antigen presentation and/or in combination of selected cytokine genes which would promote to immune potentiation (for example IL-2, IL-12, IFN-γ, TNF-α, IL-18 etc.). Such proteins include MHC molecules (Class I or Class II), CD80, CD86, or CD40. Most preferably DCs or DC-precursors are included as a source of APCs.

Dendritic cells (DCs) can be isolated/prepared by a number of means, for example they can either be purified directly from peripheral blood, or generated from $CD34^+$

precursor cells for example after mobilisation into peripheral blood by treatment with GM-CSF, or directly from bone marrow. From peripheral blood, adherent precursors can be treated with a GM-CSF/IL-4 mixture (Inaba *et al.*, 1992), or from bone marrow, non-adherent $CD34^+$ cells can be treated with GM-CSF and TNF-α (Caux *et al.*, 1992). DCs can also be routinely prepared from the peripheral blood of human volunteers, similarly to the method of Sallusto and Lanzavecchia (1994) using purified peripheral blood mononucleocytes (PBMCs) and treating 2 hour adherent cells with GM-CSF and IL-4. If required, these may be depleted of $CD19^+$ B cells and $CD3^+$, $CD2^+$ T cells using magnetic beads (see Coffin *et al.*, 1998). Culture conditions may include other cytokines such as GM-CSF or IL-4 for the maintenance and, or activity of the dendritic cells or other antigen presenting cells.

T cells

Where T cells are to be used in the *ex vivo* methods of the invention, the T cells are typically infiltrating T lymphocytes isolated from a solid tumour within the body of an individual suffering from a cancer. Alternatively other T cells such as $CD8^+$ cells may be used. It may also be convenient to use cell lines such as T cell hybridomas. However, where T cells are used in preliminary *in vitro* screening procedures to identify and test suitable nucleic acid sequences, T cells from any suitable source, such as a healthy patient, may be used and may be obtained from blood or another source (such as lymph nodes, spleen, or bone marrow). They may optionally be enriched or purified by standard procedures. The T cells may be used in combination with other immune cells, obtained from the same or a different individual. Alternatively whole blood may be used or leukocyte enriched blood or purified white blood cells as a source of T cells and other cell types. It is particularly preferred to use helper T cells ($CD4^+$).

Lymphocytes with antigen receptors recognising antigens presented by tumour cells (tumour-reactive lymphocytes (TRLs)) can be isolated from peripheral blood, lymph nodes or from tumour tissue (tumour-infiltrating lymphocytes (TILs)). Methods for isolating and culturing TRLs are well known in the art. See for example Vose *et al.* (1977). TILs and other TRLs may be isolated and expanded in culture in the presence

of cytokines such as Interleukin (IL)-2, IL-12, IFN-γ, TNF-α, IL-18 as described by Belldegrun *et al.* (1988); Belldegrun *et al.* (1989); and Spiess *et al.* (1987). TRLs and TILs reactive with identified tumour antigens can also be isolated using MHC Class-I and Class-II tetramer technology (Dunbar *et al.*, 1998; Romero *et al.*, 1998).

Thus, it will be understood that the term "antigen presenting cell or the like" are used herein is not intended to be limited to APCs. The skilled man will understand that any vehicle capable of presenting to the T cell population may be used, for the sake of convenience the term APCs is used to refer to all these. As indicated above, preferred examples of suitable APCs include dendritic cells, L cells, hybridomas, fibroblasts, lymphomas, macrophages, B cells or synthetic APCs such as lipid membranes.

Assays

In one embodiment of the present invention, small molecules may be screened for their ability to bind presenilin or presenilin-containing complexes or components or presenilin-containing complexes, especially human PS1 or PS2. In another embodiment, compounds may be tested for their ability to induce or repress expression of presenilin, especially human PS1 or PS2, . In another embodiment they may be tested for their ability to induce or repress activity of presenilin-dependent γ-secretase. Synthetic peptide substances, including for example those derived from targets of presenilin-dependent γ-secretase activity such as amyloid precursor protein (APP) may be used in assays to detect modulators. These and other embodiments are described below.

Identification of Presenilin or Presenilin-dependent gamma-secretase modulators

The assay of the present invention is set up to detect either inhibition or enhancement of presenilin or presenilin-dependent gamma-secretase expression and/or activity in cells of the immune system by candidate compounds. The compounds may be small molecules, proteins, antibodies or other ligands. Amounts or activity of presenilin or presenilin-dependent gamma-secretase will be measured for each compound tested using standard assay techniques and appropriate controls. Preferably the detected signal is compared with a reference signal and any modulation with respect to the reference

signal measured. The assay may also be run in the presence of a known antagonist of presenilin or presenilin-dependent gamma-secretase in order to identify compounds capable of rescuing presenilin or presenilin-dependent gamma-secretase activity and/or expression, respectively.

Expression and/or activity of presenilin or presenilin-dependent gamma-secretase will be measured in proportion to cleavage of Notch Intracellular Domain (NICD) or in proportion to levels of activity or expression of downstream components of the NICD signalling pathway. Such components will be referred to as "targets" of the NICD pathway. Known targets include Deltex, Hes-1, E(spl), IL-10, CD-23, Dlx-1, CTLA4, CD-4, Numb, Mastermind and Dsh.

Any one or more of appropriate targets - such as an amino acid sequence and/or nucleotide sequence - may be used for identifying a compound capable of modulating presenilin or presenilin-dependent gamma-secretase activity and/or expression in cells of the immune system in any of a variety of drug screening techniques. The target employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly.

The assay of the present invention may be a screen, whereby a number of agents are tested. In one aspect, the assay method of the present invention is a high throughput screen.

Techniques for drug screening may be based on the method described in Geysen, European Patent Application 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesised on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with a suitable target or fragment thereof and washed. Bound entities are then detected - such as by appropriately adapting methods well known in the art. A purified target can also be coated directly onto plates for use in a drug screening techniques. Alternatively, non-neutralising antibodies can be used to capture the peptide and immobilise it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralising antibodies capable of binding a target specifically compete with a test compound for binding to a target.

Another technique for screening provides for high throughput screening (HTS) of agents having suitable binding affinity to the substances and is based upon the method described in detail in WO-A-84/03564.

It is expected that the assay methods of the present invention will be suitable for both small and large-scale screening of test compounds as well as in quantitative assays.

Various nucleic acid assays are known. Any conventional technique which is known or which is subsequently disclosed may be employed. Examples of suitable nucleic acid assay are mentioned below and include amplification, PCR, RT-PCR, RNase protection, blotting, spectrometry, reporter gene assays, gene chip arrays and other hybridization methods.

Gene presence, amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA, dot blotting (DNA or RNA analysis), or in situ hybridisation, using an appropriately labelled probe. Those skilled in the art will readily envisage how these methods may be modified, if desired.

PCR was originally developed as a means of amplifying DNA from an impure sample. The technique is based on a temperature cycle which repeatedly heats and cools the reaction solution allowing primers to anneal to target sequences and extension of those primers for the formation of duplicate daughter strands. RT-PCR uses an RNA template for generation of a first strand cDNA with a reverse transcriptase. The cDNA is then amplified according to standard PCR protocol. Repeated cycles of synthesis and denaturation result in an exponential increase in the number of copies of the target DNA produced. However, as reaction components become limiting, the rate of amplification decreases until a plateau is reached and there is little or no net increase in PCR product.

The higher the starting copy number of the nucleic acid target, the sooner this "end-point" is reached.

Real-time PCR uses probes labeled with a fluorescent tag or fluorescent dyes and differs from end-point PCR for quantitative assays in that it is used to detect PCR products as they accumulate rather than for the measurement of product accumulation after a fixed number of cycles. The reactions are characterized by the point in time during cycling when amplification of a target sequence is first detected through a significant increase in fluorescence.

The ribonuclease protection (RNase protection) assay is an extremely sensitive technique for the quantitation of specific RNAs in solution . The ribonuclease protection assay can be performed on total cellular RNA or poly(A)-selected mRNA as a target. The sensitivity of the ribonuclease protection assay derives from the use of a complementary *in vitro* transcript probe which is radiolabeled to high specific activity. The probe and target RNA are hybridized in solution, after which the mixture is diluted and treated with ribonuclease (RNase) to degrade all remaining single-stranded RNA. The hybridized portion of the probe will be protected from digestion and can be visualized via electrophoresis of the mixture on a denaturing polyacrylamide gel followed by autoradiography. Since the protected fragments are analyzed by high resolution polyacrylamide gel electrophoresis, the ribonuclease protection assay can be employed to accurately map mRNA features. If the probe is hybridized at a molar excess with respect to the target RNA, then the resulting signal will be directly proportional to the amount of complementary RNA in the sample.

PCR technology as described e.g. in section 14 of Sambrook et al., 1989, requires the use of oligonucleotide probes that will hybridise to nucleic acid. Strategies for selection of oligonucleotides are described below.

As used herein, a probe is e.g. a single-stranded DNA or RNA that has a sequence of nucleotides that includes between 10 and 50, preferably between 15 and 30 and most preferably at least about 20 contiguous bases that are the same as (or the complement of) an equivalent or greater number of contiguous bases. The nucleic acid sequences

selected as probes should be of sufficient length and sufficiently unambiguous so that false positive results are minimised. The nucleotide sequences are usually based on conserved or highly homologous nucleotide sequences or regions of polypeptides. The nucleic acids used as probes may be degenerate at one or more positions.

Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences predicted to encode ligand binding sites, and the like. For example, either the full-length cDNA clone disclosed herein or fragments thereof can be used as probes. Preferably, nucleic acid probes of the invention are labelled with suitable label means for ready detection upon hybridisation. For example, a suitable label means is a radiolabel. The preferred method of labelling a DNA fragment is by incorporating α^{32}P dATP with the Klenow fragment of DNA polymerase in a random priming reaction, as is well known in the art. Oligonucleotides are usually end-labelled with γ^{32}P-labelled ATP and polynucleotide kinase. However, other methods (e.g. non-radioactive) may also be used to label the fragment or oligonucleotide, including e.g. enzyme labelling, fluorescent labelling with suitable fluorophores and biotinylation.

Preferred are such sequences, probes which hybridise under high-stringency conditions.

Stringency of hybridisation refers to conditions under which polynucleic acids hybrids are stable. Such conditions are evident to those of ordinary skill in the field. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (Tm) of the hybrid which decreases approximately 1 to 1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridisation reaction is performed under conditions of higher stringency, followed by washes of varying stringency.

As used herein, high stringency refers to conditions that permit hybridisation of only those nucleic acid sequences that form stable hybrids in 1 M Na+ at 65-68 °C. High stringency conditions can be provided, for example, by hybridisation in an aqueous solution containing 6x SSC, 5x Denhardt's, 1 % SDS (sodium dodecyl sulphate), 0.1

Na+ pyrophosphate and 0.1 mg/ml denatured salmon sperm DNA as non specific competitor. Following hybridisation, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridisation temperature in 0.2 - 0.1x SSC, 0.1 % SDS.

It is understood that these conditions may be adapted and duplicated using a variety of buffers, e.g. formamide-based buffers, and temperatures. Denhardt's solution and SSC are well known to those of skill in the art as are other suitable hybridisation buffers (see, e.g. Sambrook, et al., eds. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York or Ausubel, et al., eds. (1990) Current Protocols in Molecular Biology, John Wiley & Sons, Inc.). Optimal hybridisation conditions have to be determined empirically, as the length and the GC content of the hybridising pair also play a role.

Gene expression may also be detected using a reporter system. Such a reporter system may comprise a readily identifiable marker under the control of an expression system, e.g. of the gene being monitored. Fluorescent markers, which can be detected and sorted by FACS, are preferred. Especially preferred are GFP and luciferase. Another type of preferred reporter is cell surface markers, i.e. proteins expressed on the cell surface and therefore easily identifiable.

In general, reporter constructs useful for detecting Notch signalling by expression of a reporter gene may be constructed according to the general teaching of Sambrook et al (1989). Typically, constructs according to the invention comprise a promoter by the gene of interest, and a coding sequence encoding the desired reporter constructs, for example of GFP or luciferase. Vectors encoding GFP and luciferase are known in the art and available commercially. In a preferred embodiment, the 5' regulatory region of a presenilin gene, especially human PS1 or PS2, or presenilin-dependent gamma-secretase gene is operatively joined to a reporter gene and cells are transformed with this recombinant construct. Such recombinant cells may then be used in high through-put assays for compounds which affect the expression of presenilin or presenilin-dependent gamma-secretase.

Sorting of cells, based upon detection of expression of genes, may be performed by any technique known in the art, as exemplified above. For example, cells may be sorted by flow cytometry or FACS. For a general reference, see Flow Cytometry and Cell Sorting: A Laboratory Manual (1992) A. Radbruch (Ed.), Springer Laboratory, New York.

Flow cytometry is a powerful method for studying and purifying cells. It has found wide application, particularly in immunology and cell biology: however, the capabilities of the FACS can be applied in many other fields of biology. The acronym F.A.C.S. stands for Fluorescence Activated Cell Sorting, and is used interchangeably with "flow cytometry". The principle of FACS is that individual cells, held in a thin stream of fluid, are passed through one or more laser beams, causing light to be scattered and fluorescent dyes to emit light at various frequencies. Photomultiplier tubes (PMT) convert light to electrical signals, which are interpreted by software to generate data about the cells. Sub-populations of cells with defined characteristics can be identified and automatically sorted from the suspension at very high purity (~100%).

FACS can be used to measure gene expression in cells transfected with recombinant DNA encoding polypeptides. This can be achieved directly, by labelling of the protein product, or indirectly by using a reporter gene in the construct. Examples of reporter genes are β-galactosidase and Green Fluorescent Protein (GFP). β-galactosidase activity can be detected by FACS using fluorogenic substrates such as fluorescein digalactoside (FDG). FDG is introduced into cells by hypotonic shock, and is cleaved by the enzyme to generate a fluorescent product, which is trapped within the cell. One enzyme can therefore generate a large amount of fluorescent product. Cells expressing GFP constructs will fluoresce without the addition of a substrate. Mutants of GFP are available which have different excitation frequencies, but which emit fluorescence in the same channel. In a two-laser FACS machine, it is possible to distinguish cells which are excited by the different lasers and therefor assay two transfections at the same time.

Alternative means of cell sorting may also be employed. For example, the invention comprises the use of nucleic acid probes complementary to mRNA. Such probes can

be used to identify cells expressing polypeptides individually, such that they may subsequently be sorted either manually, or using FACS sorting. Nucleic acid probes complementary to mRNA may be prepared according to the teaching set forth above, using the general procedures as described by Sambrook et al (1989).

In a preferred embodiment, the invention comprises the use of an antisense nucleic acid molecule, complementary to a mRNA, conjugated to a fluorophore which may be used in FACS cell sorting.

Methods have also been described for obtaining information about gene expression and identity using so-called gene chip arrays or high density DNA arrays (Chee). These high density arrays are particularly useful for diagnostic and prognostic purposes. Use may also be made of In Vivo Expression Technology (IVET) (Camilli). IVET identifies genes up-regulated during say treatment or disease when compared to laboratory culture.

The advantage of using a protein assay is that Notch activation can be directly measured. Assay techniques that can be used to determine levels of a polypeptide are well known to those skilled in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection, FACS and ELISA assays.

The invention, in certain embodiments, includes antibodies specifically recognising and binding to polypeptides.

Antibodies may be recovered from the serum of immunised animals. Monoclonal antibodies may be prepared from cells from immunised animals in the conventional manner.

The antibodies of the invention are useful for identifying cells expressing the genes being monitored.

Antibodies according to the invention may be whole antibodies of natural classes, such as IgE and IgM antibodies, but are preferably IgG antibodies. Moreover, the invention includes antibody fragments, such as Fab, F(ab')2, Fv and ScFv. Small fragments, such Fv and ScFv, possess advantageous properties for diagnostic and therapeutic applications on account of their small size and consequent superior tissue distribution.

The antibodies may comprise a label. Especially preferred are labels which allow the imaging of the antibody in neural cells in vivo. Such labels may be radioactive labels or radioopaque labels, such as metal particles, which are readily visualisable within tissues. Moreover, they may be fluorescent labels or other labels which are visualisable in tissues and which may be used for cell sorting.

For assays involving monitoring or detection of tolerised T cells for use in clinical applications, the assay will generally involve removal of a sample from a patient prior to the step of detecting a signal resulting from cleavage of the intracellular domain.

The invention additionally provides a method of screening for a candidate modulator of presenilin or presenilin-dependent gamma-secretase activity and/or expression, the method comprising mixing in a buffer an appropriate amount of presenilin or presenilin-dependent gamma-secretase together with an appropriate amount of Notch, wherein Notch is suitably labelled with detection means for monitoring cleavage of Notch; and a sample of a candidate ligand; and monitoring any cleavage of Notch.

As used herein, the term "sample" refers to a collection of inorganic, organic or biochemical molecules which is either found in nature (e.g., in a biological- or other specimen) or in an artificially-constructed grouping, such as agents which may be found and/or mixed in a laboratory. The biological sample may refer to a whole organism, but more usually to a subset of its tissues, cells or component parts (e.g. body fluids, including but not limited to blood, mucus, saliva and urine).

Further methods of identifying presenilin or presenilin-dependent gamma-secretase modulators are described below. Presenilin or presenilin-dependent gamma-secretase modulators could be, for example, enzymes, co-receptors, ligands or stabilisers. The interaction of these compounds with presenilin or presenilin-dependent gamma-secretase will be indicative of a modulating function. Assays for the detection and/or analysis of such interactions are therefore included within the scope of the invention.

Soluble recombinant presenilin or presenilin-dependent gamma-secretase fusion proteins can be made, or the nucleotide sequence coding for presenilin or presenilin-dependent gamma-secretase amino acids (in particular functional domain amino acids) can be expressed, in suitable vectors (yeast-2-hybrid, baculovirus, and phage-display systems for instance) and used to identify proteins which interact with PS1 or PS2. Therapies can be designed to modulate these interactions and thus to modulate diseases of the immune system and other conditions associated with acquired or inherited abnormalities of the PS1 or PS2 genes or their gene products. The potential efficacy of these therapies can be tested by analyzing the affinity and function of these interactions after exposure to the therapeutic agent by standard pharmacokinetic measurements of affinity (Kd and Vmax etc) using synthetic peptides or recombinant proteins corresponding to functional domains of the PS1 gene, the PS2 gene or other presenilin or presenilin-dependent gamma-secretase homologues.

Another method for assaying the effect of any interactions involving functional domains such as the hydrophilic loop of the presenilin protein is to monitor changes in the intracellular trafficking and post-translational modification of the relevant genes by in situ hybridization, immunohistochemistry, Western blotting and metabolic pulse-chase labelling studies in the presence of, and in the absence of, the therapeutic agents. A further method is to monitor the effects of "downstream" events including (i) changes in the intracellular metabolism, trafficking and targeting of APP and its products; (ii) changes in second messenger events, e.g., cAMP intracellular Ca^{++} protein kinase activities, etc.

Four domains have been identified as providing functional specificity to the presenilins. These functional domains are (1) the N-terminus (unique sequence in PS1 and PS2); (2) the TM6→7 loop (clustered mutations in the flanking conserved

hydrophobic sequences and unique internal sequence); (3) the TM1, TM2 domains and TM1→2 linking sequence (concentration of several familial AD mutations) and (4) the C-terminus. To isolate proteins that interact with these functional domains, screening for presenilin binding proteins is carried out using GST-fusion constructs and synthetic peptides corresponding to these regions. For example, for PS2, GST-fusion peptides are made including sequences corresponding to amino acids 1 to 87 (N-terminus) or 272-390 (TM6→7 loop) or a synthetic peptide is made corresponding to amino acids 107 to 134 (TM1→2 link); for PS1, GST-fusion peptides are made including sequences corresponding to amino acids 1 to 81 (N-terminus) or 266 to 410 (TM6→7 loop) or a synthetic peptide is made corresponding to amino acids 101 to 131 (TM1→2 link). The following methods may be employed to isolate presenilin or presenilin-dependent gamma-secretase binding proteins:

(1) direct extraction by affinity chromatography using GST-fusion proteins and synthetic peptides;

(2) co-isolation of presenilins and bound proteins by immunoprecipitation;

(3) Biomolecular Interaction Assay (BIAcore) utilizing a GST-fusion capture system; and

(4) Two-Hybrid yeast systems.

GST-fusion proteins containing the N-terminus and TM6→7 loop sequences for PS1 and PS2 are used to probe human patient tissues and the isolated collection of proteins is separated by SDS-PAGE and microsequenced (Phizicky and Fields, 1995). To ensure that the band being sequenced contains only one protein species, the presenilin-fusion or presenilin-dependent gamma-secretase-fusion and binding proteins are separated by 2D gel electrophoresis prior to transfer and sequencing. For proteins with a blocked N-terminus, an additional HPLC purification and cleavage (CNBr and/or trypsin) of the particular binding protein is used to release peptide fragments. Further purification by HPLC and microsequencing by conventional methods provides internal sequence data on such blocked proteins.

The TM1→2 linking sequence is predicted to reside on the opposite side of the membrane to that of the N-terminal and TM6→7 loop and may be important in transmembrane communication. This is supported by the Tyr115His mutation which was observed in a pedigree with early onset familial AD (30-40 years) and by additional mutations in the TM1/2 helices which might be expected to destabilise the loop. The TM1→2 loop is relatively short (PS1: residues 101-131; PS2: residues 107-134) making this sequence more amenable to conventional peptide synthesis. The PS1 fragment (31-mer) has been synthesised containing an additional C-terminal cysteine residue. This peptide will be used to create an affinity substrate for affinity chromatography (Sulfo-link; Pierce) to isolate binding proteins for microsequencing. A peptide corresponding to the PS2 sequence is similarly synthesised and used to screen for distinct binding proteins.

An additional technique for the isolation of presenilins or presenilin-dependent gamma-secretases and their associated proteins is direct immunoprecipitation with antibodies. This procedure has been successfully used, for example, to isolate many of the synaptic vesicle associated proteins.

A useful method for the detection and isolation of binding proteins is the BIAcore system developed by Pharmacia Biosensor and described in the manufacturer's protocol (LKB Pharmacia, Sweden). This system uses an affinity purified anti-GST antibody to immobilise GST-fusion proteins onto a sensor chip. The sensor uses surface plasmon resonance which is an optical phenomenon that detects changes in refractive indices. A homogenate of a tissue of interest is passed over the immobilised fusion protein and protein-protein interactions are registered as changes in the refractive index. This system can be used to determine the kinetics of binding, to assess whether any observed binding is of physiological relevance.

The Two-Hybrid system takes advantage of transcriptional factors that are composed of two physically separable, functional domains (Fields and Sternglanz). The most commonly used is the yeast GAL4 transcriptional activator consisting of a DNA binding domain and a transcriptional activation domain. Two different cloning vectors are used to generate separate fusions of the GAL4 domains to genes encoding potential binding proteins. The fusion proteins are co-expressed, targeted to the

nucleus and, if interactions occur, activation of a reporter gene (e.g. lacZ) produces a detectable phenotype. For example, the Clontech Matchmaker System-2 may be used to screen the Clontech cDNA GAL4 activation domain fusion library with presenilin GAL4 binding domain fusion clones (Clontech, Palo Alto, Calif.).

Small molecule-based therapies are particularly preferred because such molecules are more readily absorbed after oral administration and/or have fewer potential antigenic determinants than larger, protein-based pharmaceuticals. In light of the present disclosure, one of ordinary skill in the art will be able to develop drug screening methodologies which will be useful in the identification of candidate small molecule pharmaceuticals for the treatment of immune diseases. In particular, the skilled person will be able to screen large libraries of small molecules in order to identify those which bind to the normal and/or mutant PS1 or PS2 protein and which, therefore, are candidates for modifying the in vivo activity of the normal or mutant presenilin or presenilin-dependent gamma-secretase proteins. Furthermore, the skilled person will be able to identify small molecules which selectively or preferentially bind to a mutant form of a presenilin protein or presenilin-dependent gamma-secretase.

Methods for screening small molecule libraries for candidate protein-binding molecules are well known in the art and, in light of the present disclosure, may now be employed to identify compounds which bind to the normal or mutant forms of a presenilin or presenilin-dependent gamma-secretase.

Briefly, in one embodiment, either a normal or mutant PS1 or PS2 protein may be immobilised on a substrate such as a column or filter, and a solution including the test compound(s) is contacted with the presenilin protein under conditions which are permissive for binding. The substrate is then washed with a solution which substantially reflects physiological conditions to remove unbound or weakly bound small molecules. A second wash may then elute those compounds which strongly bound to the immobilised normal or mutant presenilin. Alternatively, the small molecule test compounds may be immobilised and a solution of normal or mutant PS1 or PS2 may be contacted with the column, filter or other substrate. The ability of the presenilin to bind to the small molecules may be determined as above or a labelled

form of presenilin (e.g., radio-labelled or chemiluminescent) may be used to more rapidly assess binding to the substrate-immobilised compound(s).

In addition, as both PS1 and PS2 are believed to be membrane associated proteins, it may be preferred that the presenilin proteins be incorporated into lipid bilayers (e.g., liposomes) to promote their proper folding. Such presenilin-liposomes may be immobilised on substrates (either directly or by means of another element in the liposome membrane), passed over substrates with immobilised small molecules, or used in any of a variety of other well known binding assays for membrane proteins. In another series of embodiments, either normal or mutant, free or membrane-bound PS1 or PS2 may be mixed in a solution with the candidate compound(s) under conditions which are permissive for binding, and the presenilin may be immunoprecipitated. Small molecules which co-immunoprecipitate with a presenilin may then be identified. As will be obvious to one of ordinary skill in the art, there are numerous other methods of screening individual small molecules or large libraries of small molecules (e.g., phage display libraries) to identify compounds which bind to normal or mutant presenilins or presenilin-dependent gamma-secretase. All of these methods comprise the step of mixing normal or mutant presenilin or presenilin-dependent gamma-secretase with test compounds, allowing for binding (if any), and assaying for bound complexes.

Compounds which bind to normal or mutant or both forms of presenilins or presenilin-dependent gamma-secretase may have utility in treatments. Compounds which bind only to a normal presenilin or presenilin-dependent gamma-secretase may, for example, act as enhancers of its normal activity and thereby at least partially compensate for the lost or abnormal activity of mutant forms of the presenilin or presenilin-dependent gamma-secretase in patients suffering from immune diseases. Compounds which bind to both normal and mutant forms of a presenilin or presenilin-dependent gamma-secretase may have utility if they differentially affect the activities of the two forms so as to alleviate the overall departure from normal function.

Alternatively, blocking the activity of both normal and mutant forms of either PS1 or PS2 in heterozygotes may have less severe physiological and clinical consequences than the normal progress of the disease and, therefore, compounds which bind to and

inhibit the activity of both normal and mutant forms of a presenilin may have utility. Preferably, however, compounds are identified which have a higher affinity of binding to mutant presenilin than to normal presenilin (e.g., 5-10 fold higher K_a) and which selectively or preferentially inhibit the activity of the mutant form. Such compounds may be identified by using any of the techniques described above and by then comparing the binding affinities of the candidate compound(s) for the normal and mutant forms of PS1 or PS2.

Once identified by the methods described above, the candidate compounds may then be produced in quantities sufficient for pharmaceutical administration or testing or may serve as "lead compounds" in the design and development of new pharmaceuticals. For example, as in well known in the art, sequential modification of small molecules (e.g., amino acid residue replacement with peptides; functional group replacement with peptide or non-peptide compounds) is a standard approach in the pharmaceutical industry for the development of new pharmaceuticals. Such development generally proceeds from a "lead compound" which is shown to have at least some of the activity (e.g., PS1 binding ability) of the desired pharmaceutical. In particular, when one or more compounds having at least some activity of interest (e.g., PS1 binding) are identified, structural comparison of the molecules can greatly inform the skilled practitioner by suggesting portions of the lead compounds which should be conserved and portions which may be varied in the design of new candidate compounds. Thus, the present invention also provides a means of identifying lead compounds which may be sequentially modified to produce new candidate compounds for use in the treatment of immune disease. These new compounds then may be tested both for presenilin-binding or presenilin-dependent gamma-secretase-binding (e.g., in the binding assays described above) and for therapeutic efficacy (e.g., in the animal models described herein). This procedure may be iterated until compounds having the desired therapeutic activity and/or efficacy are identified.

In another series of embodiments, the present invention provides assays for identifying small molecules or other compounds which are capable of inducing or inhibiting the expression of PS1, PS2 or other presenilin-related or presenilin-dependent gamma-secretase-related genes and proteins. The assays may be performed in vitro using non-transformed cells, immortalised cell lines, or recombinant cell lines.

In addition, the assays may detect the presence of increased or decreased expression of PS1 , PS2 or other presenilin-related or presenilin-dependent gamma-secretase-related genes or proteins on the basis of increased or decreased mRNA expression (using, e.g., the nucleic acid probes disclosed and enabled herein), increased or decreased levels of PS1, PS2 or other presenilin-related or presenilin-dependent gamma-secretase-related protein products (using, e.g., the anti-presenilin or presenilin-dependent gamma-secretase antibodies disclosed and enabled herein), or increased or decreased levels of expression of a reporter gene (e.g., β-galactosidase or luciferase) operatively joined to a presenilin or presenilin-dependent gamma-secretase 5' regulatory region in a recombinant construct.

Thus, for example, one may culture cells known to express a particular presenilin or presenilin-dependent gamma-secretase and add to the culture medium one or more test compounds. After allowing a sufficient period of time (e.g., 6-72 hours) for the compound to induce or inhibit the expression of the presenilin or presenilin-dependent gamma-secretase, any change in levels of expression from an established baseline may be detected using any of the techniques described above and well known in the art. In particularly preferred embodiments, the cells are from an immortalised cell line such as a human glioblastoma cell line or a hybridoma-glioma cell line. Using the nucleic acid probes and/or antibodies disclosed and enabled herein, detection of changes in the expression of a presenilin or presenilin-dependent gamma-secretase, and thus identification of the compound as an inducer or repressor of presenilin or presenilin-dependent gamma-secretase expression, requires only routine experimentation.

In particularly preferred embodiments, a recombinant assay is employed in which a reporter gene such a β-galactosidase or luciferase is operably joined to the 5' regulatory regions of a presenilin or presenilin-dependent gamma-secretase gene. Such regulatory regions may be easily isolated and cloned by one of ordinary skill in the art in light of the present disclosure of the coding regions of these genes. The reporter gene and regulatory regions are joined in-frame (or in each of the three possible reading frames) so that transcription and translation of the reporter gene may proceed under the control of the presenilin or presenilin-dependent gamma-secretase regulatory elements. The recombinant construct may then be introduced into any

appropriate cell type although mammalian cells are preferred, and human cells are most preferred. The transformed cells may be grown in culture and, after establishing the baseline level of expression of the reporter gene, test compounds may be added to the medium. The ease of detection of the expression of the reporter gene provides for a rapid, high through-put assay for the identification of inducers and repressors of the presenilin or presenilin-dependent gamma-secretase gene.

Compounds identified by this method will have potential utility in modifying the expression of the PS1, PS2 or other presenilin-related or presenilin-dependent gamma-secretase-related genes in vivo. These compounds may be further tested in the animal models disclosed and enabled herein to identify those compounds having the most potent in vivo effects. In addition, as described above with respect to small molecules having presenilin-binding or presenilin-dependent gamma-secretase-binding activity, these molecules may serve as "lead compounds" for the further development of pharmaceuticals by, for example, subjecting the compounds to sequential modifications, molecular modelling, and other routine procedures employed in rational drug design.

Candidate compounds

The compound of the invention may be an organic compound or other chemical. In one preferred embodiment, the compound will be an amino acid sequence or a chemical derivative thereof, or a combination thereof. In another preferred embodiment, the compound will be a nucleotide sequence - which may be a sense sequence or an anti-sense sequence. The compound may also be an antibody.

Alternatively, the compound will be an organic compound comprising two or more hydrocarbyl groups. Here, the term "hydrocarbyl group" means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element

or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen. The compound may comprise at least one cyclic group. The cyclic group may be a polycyclic group, such as a non-fused polycyclic group. For some applications, the agent comprises at least the one of said cyclic groups linked to another hydrocarbyl group.

Polypeptides and Polynucleotides

Amino Acid Sequences

As used herein, the term "amino acid sequence" is synonymous with the term "polypeptide" and/or the term "protein". In some instances, the term "amino acid sequence" is synonymous with the term "peptide". In some instances, the term "amino acid sequence" is synonymous with the term "protein".

"Peptide" usually refers to a short amino acid sequence that is 10 to 40 amino acids long, preferably 10 to 35 amino acids.

The amino acid sequence may be prepared and isolated from a suitable source, or it may be made synthetically or it may be prepared by use of recombinant DNA techniques.

Nucleotide Sequences

As used herein, the term "nucleotide sequence" is synonymous with the term "polynucleotide".

The nucleotide sequence may be DNA or RNA of genomic or synthetic or of recombinant origin. They may also be cloned by standard techniques. The nucleotide sequence may be double-stranded or single-stranded whether representing the sense or antisense strand or combinations thereof.

Longer nucleotide sequences will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction (PCR) under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector. In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

"Polynucleotide" refers to a polymeric form of nucleotides of at least 10 bases in length and up to 1,000 bases or even more, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

These may be constructed using standard recombinant DNA methodologies. The nucleic acid may be RNA or DNA and is preferably DNA. Where it is RNA, manipulations may be performed via cDNA intermediates. Generally, a nucleic acid sequence encoding the first region will be prepared and suitable restriction sites provided at the 5' and/or 3' ends. Conveniently the sequence is manipulated in a standard laboratory vector, such as a plasmid vector based on pBR322 or pUC19 (see below). Reference may be made to Molecular Cloning by Sambrook *et al.* (Cold Spring Harbor, 1989) or similar standard reference books for exact details of the appropriate techniques.

Sources of nucleic acid may be ascertained by reference to published literature or databanks such as GenBank. Nucleic acid encoding the desired first or second sequences may be obtained from academic or commercial sources where such sources are willing to provide the material or by synthesising or cloning the appropriate

sequence where only the sequence data are available. Generally this may be done by reference to literature sources which describe the cloning of the gene in question.

Alternatively, where limited sequence data is available or where it is desired to express a nucleic acid homologous or otherwise related to a known nucleic acid, exemplary nucleic acids can be characterised as those nucleotide sequences which hybridise to the nucleic acid sequences known in the art.

For some applications, preferably, the nucleotide sequence is DNA. For some applications, preferably, the nucleotide sequence is prepared by use of recombinant DNA techniques (e.g. recombinant DNA). For some applications, preferably, the nucleotide sequence is cDNA. For some applications, preferably, the nucleotide sequence may be the same as the naturally occurring form.

The nucleotide sequence may comprise, for example, a protein-encoding domain, an antisense sequence or a functional motif such as a protein-binding domain and includes variants, derivatives, analogues and fragments thereof. The term also refers to polypeptides encoded by the nucleotide sequence.

Variants, Derivatives, Analogues, Homologues and Fragments

In addition to the specific amino acid sequences and nucleotide sequences mentioned herein, the present invention also encompasses the use of variants, derivatives, analogues, homologues and fragments thereof.

In the context of the present invention, a variant of any given sequence is a sequence in which the specific sequence of residues (whether amino acid or nucleic acid residues) has been modified in such a manner that the polypeptide or polynucleotide in question retains at least one of its endogenous functions. A variant sequence can be modified by addition, deletion, substitution modification replacement and/or variation of at least one residue present in the naturally-occurring protein.

The term "derivative" as used herein, in relation to proteins or polypeptides of the present invention includes any substitution of, variation of, modification of,

replacement of, deletion of and/or addition of one (or more) amino acid residues from or to the sequence providing that the resultant protein or polypeptide retains at least one of its endogenous functions.

The term "analogue" as used herein, in relation to polypeptides or polynucleotides includes any mimetic, that is, a chemical compound that possesses at least one of the endogenous functions of the polypeptides or polynucleotides which it mimics.

Typically, amino acid substitutions may be made, for example from 1, 2 or 3 to 10 or 20 substitutions provided that the modified sequence retains the required activity or ability. Amino acid substitutions may include the use of non-naturally occurring analogues.

Proteins of use in the present invention may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the transport or modulation function is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

As used herein, the term "protein" includes single-chain polypeptide molecules as well as multiple-polypeptide complexes where individual constituent polypeptides are linked by covalent or non-covalent means. As used herein, the terms "polypeptide" and "peptide" refer to a polymer in which the monomers are amino acids and are joined together through peptide or disulfide bonds. The terms subunit and domain may also refer to polypeptides and peptides having biological function.

"Fragments" are also variants and the term typically refers to a selected region of the polypeptide or polynucleotide that is of interest either functionally or, for example, in an assay. "Fragment" thus refers to an amino acid or nucleic acid sequence that is a portion of a full-length polypeptide or polynucleodtide.

Such variants may be prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed in accordance with the invention to make the encoded protein. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used.

Polynucleotide variants will preferably comprise codon optimised sequences. Codon optimisation is known in the art as a method of enhancing RNA stability and therefor gene expression. The redundancy of the genetic code means that several different codons may encode the same amino-acid. For example, Leucine, Arginine and Serine are each encoded by six different codons. Different organisms show preferences in their use of the different codons. Viruses such as HIV, for instance, use a large number of rare codons. By changing a nucleotide sequence such that rare codons are replaced by the corresponding commonly used mammalian codons, increased expression of the sequences in mammalian target cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms. Preferably, at least part of the sequence is codon optimised. Even more preferably, the sequence is codon optimised in its entirety.

As used herein, the term "homology" can be equated with "identity". An homologous sequence will be taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical. In particular, homology should typically be considered with respect to those regions of the sequence (such as amino acids at positions 51, 56 and 57) known to be essential for an activity. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

Percent homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one

insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefor firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package, FASTA (Atschul) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of

such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

Nucleotide sequences which are homologous to or variants of sequences of use in the present invention can be obtained in a number of ways, for example by probing DNA libraries made from a range of sources. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of the reference nucleotide sequence under conditions of medium to high stringency. Similar considerations apply to obtaining species homologues and allelic variants of the amino acid and/or nucleotide sequences useful in the present invention.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of use in the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used. The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences.

Alternatively, such nucleotide sequences may be obtained by site directed mutagenesis of characterised sequences. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the nucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the activity of the polynucleotide or encoded polypeptide.

Immunotherapy

The modulators of the present invention including those identified by the assay method of the present invention may be used as therapeutic agents – i.e. in therapy applications.

The term "therapy" includes curative effects, alleviation effects, and prophylactic effects. The therapy may be on humans or animals.

Such modulators of the present invention may be used in immunotherapy, i.e. to treat disorders and/or conditions of the immune system. In particular, the compounds can be used in the treatment of T cell mediated diseases or disorders. A detailed description of the conditions affected by the Notch signalling pathway may be found in our WO98/20142, WO00/36089 and WO/00135990.

Diseased or infectious states that may be described as being mediated by T cells include, but are not limited to, any one or more of asthma, allergy, tumour induced aberrations to the T cell system and infectious diseases such as those caused by Plasmodium species, Microfilariae, Helminths, Mycobacteria, HIV, Cytomegalovirus, Pseudomonas, Toxoplasma, Echinococcus, Haemophilus influenza type B, measles, Hepatitis C or Toxicara. Thus particular conditions that may be treated or prevented which are mediated by T cells include multiple sclerosis, rheumatoid arthritis and diabetes. The present invention may also be used in organ transplantation or bone marrow transplantation. The present invention is also useful in treating immune disorders such as autoimmune disorders or graft rejection such as allograft rejection.

Examples of autoimmune disorders range from organ specific diseases (such as thyroiditis, insulitis, multiple sclerosis, iridocyclitis, uveitis, orchitis, hepatitis, Addison's disease, myasthenia gravis) to systemic illnesses such as rheumatoid arthritis or lupus erythematosus. Other disorders include immune hyperreactivity, such as allergic reactions.

In more detail, organ-specific autoimmune diseases include multiple sclerosis, insulin dependent diabetes mellitus, several forms of anemia (aplastic, hemolytic), autoimmune hepatitis, thyroiditis, insulitis, iridocyclitis, skleritis, uveitis, orchitis, myasthenia gravis, idiopathic thrombocytopenic purpura, inflammatory bowel diseases (Crohn's disease, ulcerative colitis).

Systemic autoimmune diseases include: rheumatoid arthritis, juvenile arthritis, scleroderma and systemic sclerosis, sjogren's syndrom, undifferentiated connective tissue syndrome, antiphospholipid syndrome, different forms of vasculitis (polyarteritis nodosa, allergic granulomatosis and angiitis, Wegner's granulomatosis, Kawasaki disease, hypersensitivity vasculitis, Henoch-Schoenlein purpura, Behcet's Syndrome, Takayasu arteritis, Giant cell arteritis, Thrombangiitis obliterans), lupus erythematosus, polymyalgia rheumatica, essentiell (mixed) cryoglobulinemia, Psoriasis vulgaris and psoriatic arthritis, diffus fasciitis with or without eosinophilia, polymyositis and other idiopathic inflammatory myopathies, relapsing panniculitis, relapsing polychondritis, lymphomatoid granulomatosis, erythema nodosum, ankylosing spondylitis, Reiter's syndrome, different forms of inflammatory dermatitis.

A more extensive list of disorders includes: unwanted immune reactions and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic

diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery or organ, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of

transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

The present invention is also useful in cancer therapy, particularly in diseases involving the conversion of epithelial cells to cancer. The present invention is especially useful in relation to adenocarcinomas such as: small cell lung cancer, and cancer of the kidney, uterus, prostrate, bladder, ovary, colon and breast.

The present invention thus provides a method for enhancing the reactivity of a T cell toward a tumour cell.

In more detail, the T cells, APCs and/or tumour cells prepared by the method of the invention may be administered to a patient suffering from a malignancy, the malignancy typically comprising cancerous cells that express a Notch ligand. The presence of cancerous cells that express, in particular over-express, a Notch ligand may be determined by, for example, testing using the methods described above a sample of cancerous tissue obtained from the patient.

Generally, the patient will be the same patient from whom the treated T cells, APCs and/or tumour cells originated. Examples of malignancies that may be treated include cancer of the breast, cervix, colon, rectum, endometrium, kidney, lung, ovary, pancreas, prostate gland, skin, stomach, bladder, CNS, oesophagus, head-or-neck, liver, testis, thymus or thyroid. Malignancies of blood cells, bone marrow cells, B-lymphocytes, T-lymphocytes, lymphocytic progenitors or myeloid cell progenitors may also be treated.

The tumour may be a solid tumour or a non-solid tumour and may be a primary tumour or a disseminated metastatic (secondary) tumour. Non-solid tumours include myeloma; leukaemia (acute or chronic, lymphocytic or myelocytic) such as acute myeloblastic, acute promyelocytic, acute myelomonocytic, acute monocytic, erythroleukaemia; and lymphomas such as Hodgkin's, non-Hodgkin's and Burkitt's. Solid tumours include carcinoma, colon carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, adenocarcinoma, melanoma, basal or squamous cell carcinoma, mesothelioma, adenocarcinoma, neuroblastoma, glioma, astrocytoma,

medulloblastoma, retinoblastoma, sarcoma, osteosarcoma, rhabdomyosarcoma, fibrosarcoma, osteogenic sarcoma, hepatoma, and seminoma.

The tumour may be one which presents intracellular or membrane-bound antigens including tumour-specific antigens (for example virally encoded antigens, neo-antigens such as MUC1, antibody idiotypes); antigens which are overexpressed on the surface of tumour cells; oncofoetal antigens including cancer-testis (CT) antigens; or differentiation-antigens (such as tyrosinase and melanocyte antigens). The patient may have an ongoing immune response, such as a Th1 or Th2-type immune response, to antigens on the tumour and may have detectable cytotoxic T cell (CTL) activity, NK cell activity and/or antibody responses against the tumour as determined by, for example, *in vitro* assays.

Alternatively, the APCs and/or lymphocytes of the present invention can be used to efficiently transfer infectious tolerance to a chosen antigen or antigens when transferred into a patient for the treatment of a disease characterised by inappropriate lymphocyte activity, such as Th1 or Th2 cell activity. The APCs and/or lymphocytes may thus be used to treat an ongoing immune response (such as an allergic condition or an autoimmune disease) or may be used to generate tolerance in an immunologically lymphocytes cells of the present invention may be used in therapeutic methods for both treating and preventing diseases characterised by inappropriate lymphocyte activity in animals and humans. The APCs and/or lymphocytes may be used to confer tolerance to a single antigen or to multiple antigens. Typically, APCs and/or lymphocytes are obtained from the patient or donor and primed as described above before being returned to the patient (*ex vivo* therapy).

The present invention may also be employed to produce a lymphocyte or APC having tolerance to an allergen or antigen.

Antigens and Allergens

An antigen may be any substance that can be recognised generally as foreign, by the immune system, and is generally recognised by an antigen receptor. Preferably the

antigen used in the present invention is an immunogen. An allergic response occurs when the host is re-exposed to an antigen that it has encountered previously.

The immune response to antigen is generally either cell mediated (T cell mediated killing) or humoral (antibody production via recognition of whole antigen). The pattern of cytokine production by TH cells involved in an immune response can influence which of these response types predominates: cell mediated immunity (TH1) is characterised by high IL-2 and IFNγ but low IL-4 production, whereas in humoral immunity (TH2) the pattern is low IL-2 and IFNγ but high IL-4, IL-5, IL-10. Since the secretory pattern is modulated at the level of the secondary lymphoid organ or cells, then pharmacological manipulation of the specific TH cytokine pattern can influence the type and extent of the immune response generated.

The TH1-TH2 balance refers to the relative representation of the two different forms of helper T cells. The two forms have large scale and opposing effects on the immune system. If an immune response favours TH1 cells, then these cells will drive a cellular response, whereas TH2 cells will drive an antibody-dominated response. The type of antibodies responsible for some allergic reactions is induced by TH2 cells.

The antigen or allergen used in the present invention may be a peptide, polypeptide, carbohydrate, protein, glycoprotein, or more complex material containing multiple antigenic epitopes such as a protein complex, cell-membrane preparation, whole cells (viable or non-viable cells), bacterial cells or virus/viral component. In particular, it is preferred to use antigens known to be associated with auto-immune diseases such as myelin basic protein (associated with multiple sclerosis), collagen (associated with rheumatoid arthritis), and insulin (diabetes), or antigens associated with rejection of non-self tissue such as MHC antigens. Where primed the APCs and/or T cells of the present invention are to be used in tissue transplantation procedures, antigens will be obtained from the tissue donor.

The antigen or allergen moiety may be, for example, a synthetic MHC-peptide complex i.e. a fragment of the MHC molecule bearing the antigen groove bearing an element of the antigen. Such complexes have been described in Altman *et al.*, 1996.

Preparation of Primed APCs and Lymphocytes

- Preparation of Primed APCs *ex vivo* in the absence of lymphocytes

APCs as described above are cultured in a suitable culture medium such as DMEM or other defined media, optionally in the presence of fetal calf serum. Cytokines, if present, are typically added at up to 1000 U/ml. Optimum concentrations may be determined by titration. One or more substances capable of modulating presenilin and, optionally, one or more substances capable of up-regulating or down-regulating the Notch signalling pathway are then typically added to the culture medium together with the antigen of interest. The antigen may be added before, after or at substantially the same time as the substance(s). Cells are typically incubated with the substance(s) and antigen for at least one hour, preferably at least 3 hours, at 37°C. If required, a small aliquot of cells may be tested for modulated target gene expression as described above. Alternatively, cell activity may be measured by the inhibition of T cell proliferation as described in WO98/20142. APCs transfected with a nucleic acid construct directing the expression of, for example Serrate, may be used as a control.

As discussed above, polypeptide substances may be administered to APCs by introducing nucleic acid constructs/viral vectors encoding the polypeptide into cells under conditions that allow for expression of the polypeptide in the APC. Similarly, nucleic acid constructs encoding antigens may be introduced into the APCs by transfection, viral infection or viral transduction.

The resulting APCs that show increased levels of a Notch signalling are now ready for use.

- Preparation of Regulatory T cells (and B cells) *ex vivo*

The techniques described below are described in relation to T cells, but are equally applicable to B cells. The techniques employed are essentially identical to that described for APCs alone except that T cells are generally co-cultured with the APCs. However, it

may be preferred to prepare primed APCs first and then incubate them with T cells. For example, once the primed APCs have been prepared, they may be pelleted and washed with PBS before being resuspended in fresh culture medium. This has the advantage that if, for example, it is desired to treat the T cells with a different substance(s) capable of modulating presenilin to that used with the APC, then the T cell will not be brought into contact with the different substance(s) used in the APC. Alternatively, the T cell may be incubated with a first substance (or set of substances) to modulate presenilin or presenilin-dependent gamma-secretase and, optionally, Notch signalling, washed, resuspended and then incubated with the primed APC in the absence of both the substance(s) used to modulate the APC and the substance(s) used to modulate the T cell. Alternatively, T cells may be cultured and primed in the absence of APCs by use of APC substitutes such as anti-TCR antibodies (e.g. anti-CD3) with or without antibodies to costimulatory molecules (e.g. anti-CD28) or alternatively T cells may be activated with MHC-peptide complexes (e.g. tetramers).

Incubations will typically be for at least 1 hour, preferably at least 3 or 6 hours, in suitable culture medium at 37°C. The progress of presenilin or presenilin-dependent gamma-secretase modulation may be determined for a small aliquot of cells using the methods described above. T cells transfected with a nucleic acid construct directing the expression of, for example Delta, may be used as a control. Induction of immunotolerance may be determined by subsequently challenging T cells with antigen and measuring IL-2 production compared with control cells not exposed to APCs.

Primed T cells or B cells may also be used to induce immunotolerance in other T cells or B cells in the absence of APCs using similar culture techniques and incubation times.

Pharmaceutical Compositions

The present invention provides a pharmaceutical composition comprising administering a therapeutically effective amount of the compound identified by the method of the present invention and a pharmaceutically acceptable carrier, diluent or excipients (including combinations thereof).

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Preservatives, stabilizers, dyes and even flavoring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the compound is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or

suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

Administration

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, mucosal, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration.

The term "administered" includes delivery by viral or non-viral techniques. Viral delivery mechanisms include but are not limited to adenoviral vectors, adeno-associated viral (AAV) vectos, herpes viral vectors, retroviral vectors, lentiviral vectors, and baculoviral vectors. Non-viral delivery mechanisms include lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular, intradermal, intra-articular, intrathecal, intra-peritoneal or subcutaneous route, or via the alimentary tract (for example, via the Peyers patches). Administration may also be by use of implants, e.g. subcutaneous implants as

described in WO99/44583 (Applied Vaccine Technologies Corp).

Cells and pharmaceutical comprising cells of the invention are typically administered to the patient by intramuscular, intraperitoneal or intravenous injection, or by direct injection into the lymph nodes of the patient, preferably by direct injection into the lymph nodes. Typically from 10^4 to 10^8 treated cells, preferably from 10^5 to 10^7 cells, more preferably about 10^6 cells are administered to the patient.

The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient depending on, for example, the age, weight and condition of the patient. Preferably the pharmaceutical compositions are in unit dosage form. The present invention includes both human and veterinary applications.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

Various features and embodiments of the invention will now be described with reference to the following non-limiting Example.

Example 1: Effect of MW167 on Notch Signalling

C2C12 cells were transfected with plasmid pLOR3 (mHES1-Luc reporter construct) using Effectene transfection reagent (Qiagen) for 24 hours.

A 96-well tissue culture plate was coated overnight at 4°C with 100 µl of anti-V5 monoclonal antibody (Invitrogen) diluted to 1 µg/ml in PBS. The following day the plate was washed with PBS and 100 µl of purified mDelta1-V5-His fusion protein (BD34) diluted to 2 µg/ml in PBS was added for 2 hours at room temperature. The plate was washed with PBS before adding the transfected C2C12 cells.

The transfected C2C12 cells were trypsinised, resuspended at 4.0 x 10^5 cells/ml and plated out at 100 µl /well. The γ-secretase inhibitor, MW167 (Calbiochem γ-secretase inhibitor II, Cat. No. 565755), was dissolved in DMSO at 10 mM and added to duplicate wells at a final concentration of 100 µM. Control wells were set up with an equivalent dilution of DMSO alone. The plate was placed in a CO_2 incubator at 37°C for 24 hours.

The supernatant was removed from all the wells and 100 µl of PBS was added followed by 100µl of SteadyGlo luciferase assay regent (Promega). The plate was left for 5 minutes at room temperature and then 200 µl was removed and placed into a white 96-well OptiPlate (Packard) tissue culture plate and the luminescence read in a TopCount (Packard) counter. The results are shown in Figure 4.

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CLAIMS

1. Use of a modulator of presenilin or presenilin-dependent gamma-secretase activity for the manufacture of a medicament for use in immunotherapy.

2. Use of a modulator of presenilin or presenilin-dependent gamma-secretase activity in combination with a modulator of the Notch signalling pathway for the manufacture of a medicament for use in immunotherapy.

3. Use according to claim 1 or claim 2 wherein the medicament is for use in the treatment of a T cell mediated disease or infection.

4. Use according to claim 3 wherein the T cell mediated disease or infection is any one or more of allergy, autoimmunity, graft rejection, tumour induced aberrations to the T cell and infectious diseases.

5. Use according to any preceding claim wherein the presenilin modulator is a modulator of Presenilin-1 (PS1) or Presenilin-2 (PS2).

6. Use according to any preceding claim wherein the presenilin or presenilin-dependent gamma-secretase modulator is selected from polypeptides and fragments thereof, linear peptides, cyclic peptides, and nucleic acids which encode therefor, synthetic and natural compounds including low molecular weight organic or inorganic compounds and antibodies.

7. Use according to any preceding claim wherein the modulator is an agonist of presenilin or presenilin-dependent gamma-secretase, optionally in combination with an agent capable of up-regulating the Notch signalling pathway.

8. Use according to claim 7 wherein the agonist of presenilin is a polypeptide selected from Nicastrin or ALG-3 or a nucleic acid sequence which encodes therefor.

9. Use according to claim 7 or claim 8 wherein the agent capable of up-regulating expression the Notch signalling pathway is a polypeptide selected from Notch ligands, Noggin, Chordin, Follistatin, Xnr3, FGF and derivatives, fragments, variants and homologues thereof, and immunosuppressive cytokines, or a combination thereof, or a nucleic acid sequence which encodes therefor.

10. Use according to any of claims 1-6 wherein the modulator is an antagonist of presenilin or presenilin-dependent gamma-secretase, optionally in combination with an agent capable of down-regulating the Notch signalling pathway.

11. Use according to claim 10 wherein the antagonist of presenilin is 26S proteasome or Sel 10 or a nucleic acid sequence which encodes therefor.

12. Use according to claim 10 or 11 wherein the agent capable of down-regulating the Notch signalling pathway is a polypeptide selected from a Toll-like receptor, a cytokine, a bone morphogenetic protein (BMP), a BMP receptor or an activin, or a nucleic acid sequence which encodes therefor.

13. Modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting T cell mediated disease or infection.

14. Modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting linked suppression.

15. Modulator of presenilin or presenilin-dependent gamma-secretase activity for use in affecting infectious tolerance.

16. Modulator of presenilin or presenilin-dependent gamma-secretase activity according to any one of claims 13 to 15 in combination with a modulator of the Notch signalling pathway.

17. A method for producing a lymphocyte or antigen presenting cell (APC) having tolerance to an allergen or antigen which method comprises incubating a lymphocyte or APC obtained from a human or animal patient with (i) an agonist of presenilin or

presenilin-dependent gamma-secretase and optionally an agent capable of up-regulating endogenous Notch or Notch ligand in the lymphocyte and/or APC and (ii) the allergen or antigen.

18. A method according to claim 17 for producing an APC capable of inducing T cell tolerance.

19. A method according to claim 17 or claim 18 for producing *ex vivo* a T cell having tolerance to an allergen or antigen which method comprises incubating a T cell obtained from a human or animal patient with an antigen presenting cell (APC) in the presence of (i) an agonist of presenilin or presenilin-dependent gamma-secretase and optionally an agent capable of up-regulating expression of an endogenous Notch or Notch ligand in the APC and/or T cell and (ii) the allergen or antigen.

20. A method for producing a lymphocyte or APC having tolerance to an allergen or antigen which method comprises incubating a lymphocyte or APC obtained from a human or animal patient with a lymphocyte or APC produced by the method of any one of claims 17 to 19.

21. A method according to claim 20 for producing *ex vivo* a T cell having tolerance to an allergen or antigen which method comprises incubating a T cell obtained from a human or animal patient with a T cell produced by the method of any one claims 17 to 19.

22. Use of a lymphocyte or APC produced by the method of any one of claims 17 to 21 in suppressing an immune response in a mammal to the allergen or antigen.

23. A method of treating a patient suffering from a disease characterised by inappropriate lymphocyte activity which method comprises administering to the patient a lymphocyte produced by the method of any one of claims 17 to 21.

24. A method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating a T cell from a patient having said tumour cell present in their body;

(b) exposing the T cell to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell; and

(c) re-introducing the T cell into the patient;

wherein the T cell comprises a T cell receptor specific for a tumour antigen expressed by the tumour cell.

25. A method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating an antigen presenting cell (APC) from a tumour present in the body of a patient;

(b) exposing the APC to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell when the T cell is contacted with the APC; and

(c) re-introducing the APC into the patient.

26. A method for enhancing the reactivity of a T cell toward a tumour cell which method comprises:

(a) isolating a tumour cell from a tumour present in the body of a patient;

(b) exposing the tumour cell to a modulator of presenilin or presenilin-dependent gamma-secretase activity, optionally in the presence of an agent which is capable of reducing or preventing expression or interaction of an endogenous Notch or Notch ligand in the T cell when the T cell is contacted with the tumour cell; and

(c) re-introducing the tumour cell into the patient.

27. A method according to any one of claims 24 to 26 wherein the T cell is a tumour infiltrating lymphocyte.

28. A method of vaccinating a patient against a tumour which method comprises:

(a) administering a tumour antigen expressed by the tumour to the skin of the patient; and

(b) exposing the APC present in the skin of the patient to a modulator of presenilin or presenilin-dependent gamma-secretase agent, optionally in the presence of an agent which is capable of reducing or preventing expression, interaction or processing of Notch or a Notch ligand in a T cell.

29. An assay method for modulators of presenilin or presenilin-dependent gamma-secretase activity comprising contacting a presenilin or presenilin-dependent gamma-secretase, respectively, in the presence of Notch and a modulator of the Notch signalling pathway, with a candidate compound and determining if the compound affects the Notch signalling pathway.

30. An assay method for identifying substances that affect the interaction of a presenilin interacting protein or presenilin-dependent gamma-secretase interacting protein with a presenilin protein or presenilin-dependent gamma-secretase, respectively, comprising:

(a) providing a preparation containing: a presenilin protein or presenilin-dependent gamma-secretase; a presenilin-interacting protein or presenilin-dependent gamma-secretase, respectively; and a candidate substance; and

(b) detecting whether said candidate substance affects said interaction of said presenilin-interacting protein or presenilin-dependent gamma-secretase-interacting protein with said presenilin protein or presenilin-dependent gamma-secretase.

31. An assay method according to claim 30 wherein the presenilin-interacting protein is Notch or a member of the Notch signalling pathway.

32. Use of a presenilin or presenilin-dependent gamma-secretase modulator identifiable using the assay method of any of claims 29-31 in the use or method of any one of claims 1 to 28.

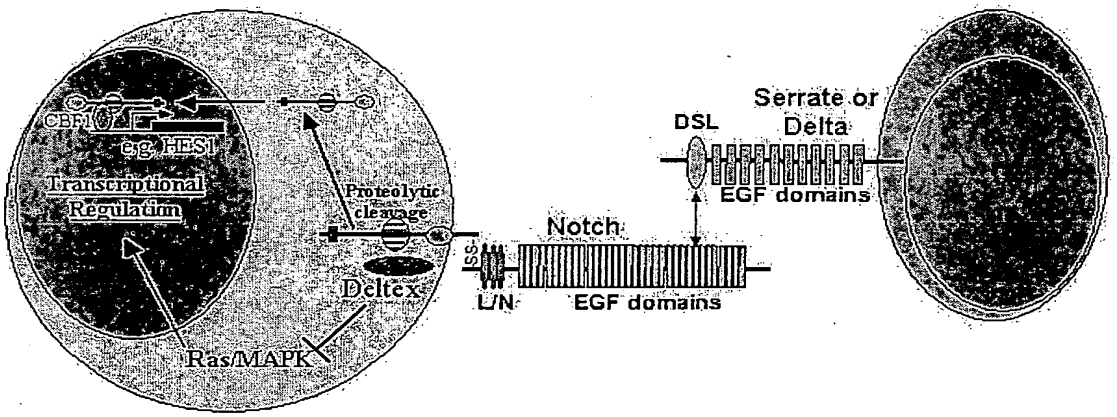
33. A kit comprising in one or more containers (a) a modulator of the Notch signalling pathway and (b) a modulator of presenilin or presenilin-dependent gamma-secretase activity.

Abstract

Modulators

Use of a modulator of presenilin or presenilin-dependent gamma-secretase activity in the manufacture of a medicament for use in immunotherapy and methods of detecting such a modulator.

Figure 1


CBF1
e.g. HES1
Transcriptional
Regulatim
Proteolytic
cleavage
Deltex
Ras/MAPK
Notch
S/S
L/N
EGF domains
DSL
Serrate or
Delta
EGF domains

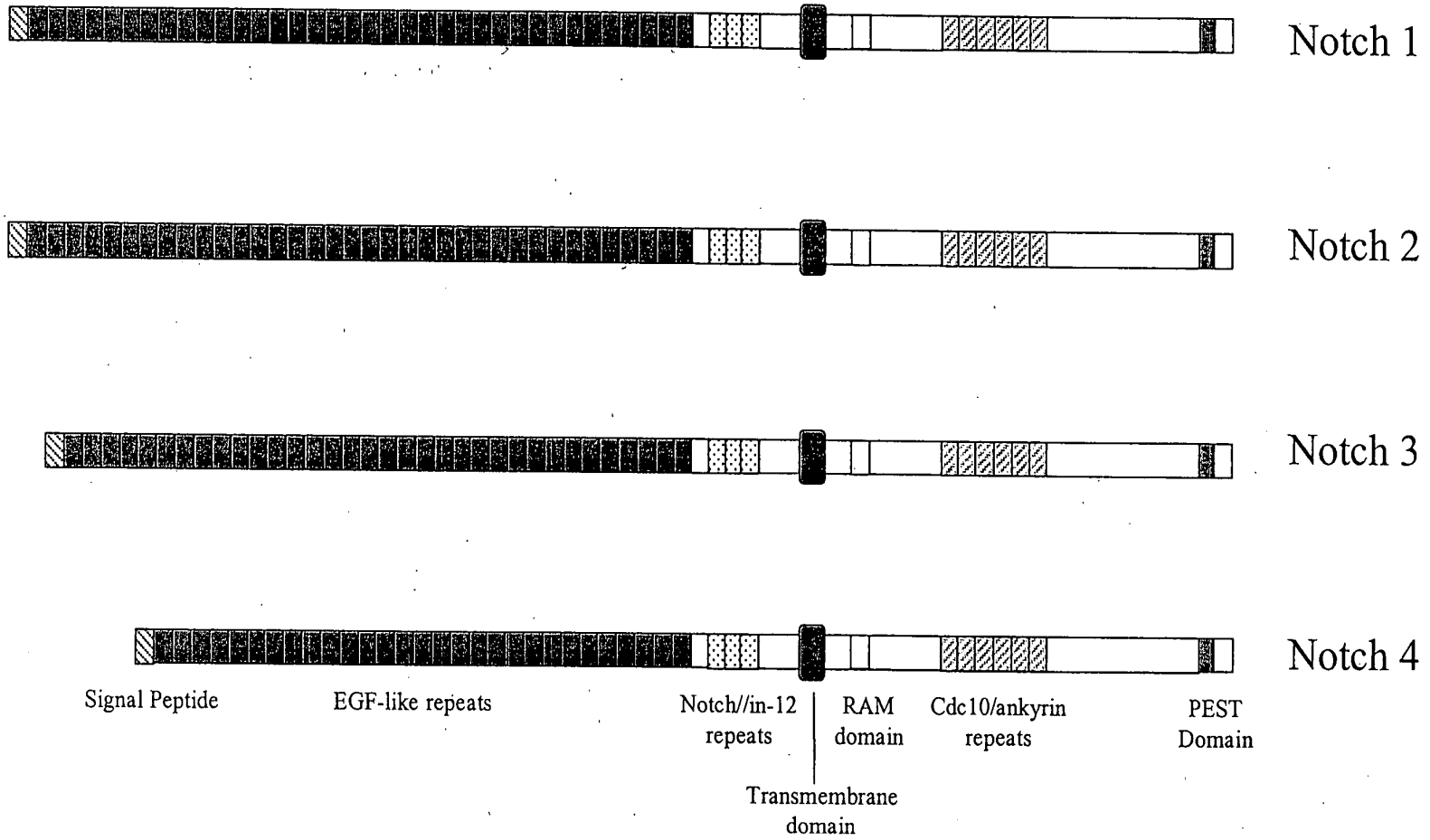
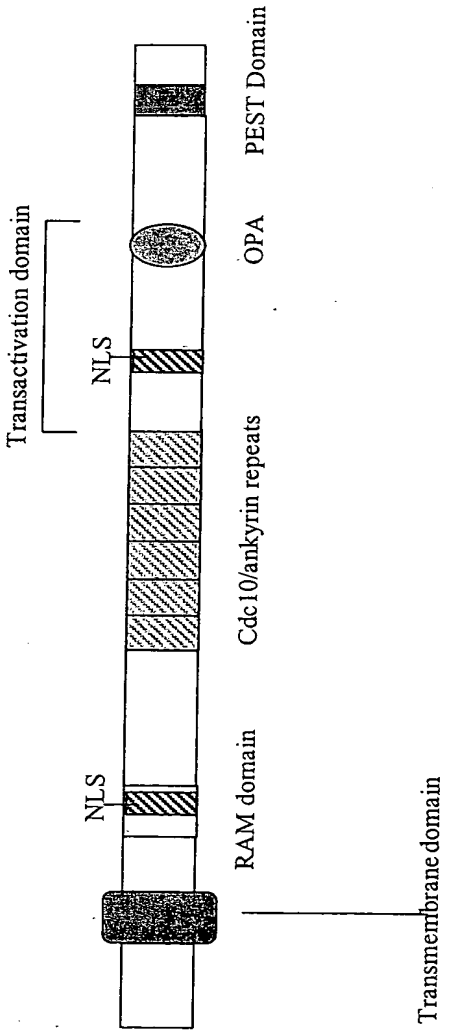

Notch 1
Notch 2
Notch 3
Notch 4
Signal Peptide
EGF-like repeats
Notch//in-12 repeats
RAM domain
Cdc10/ankyrin repeats
PEST Domain
Transmembrane domain

Figure 2

Figure 3


Transactivation domain
NLS
NLS
PEST Domain
OPA
Cdc10/ankyrin repeats
RAM domain
Transmembrane domain

Effect of MW167 on Notch Signalling in C2C12 Cells Transfected with mHES1-Luciferase

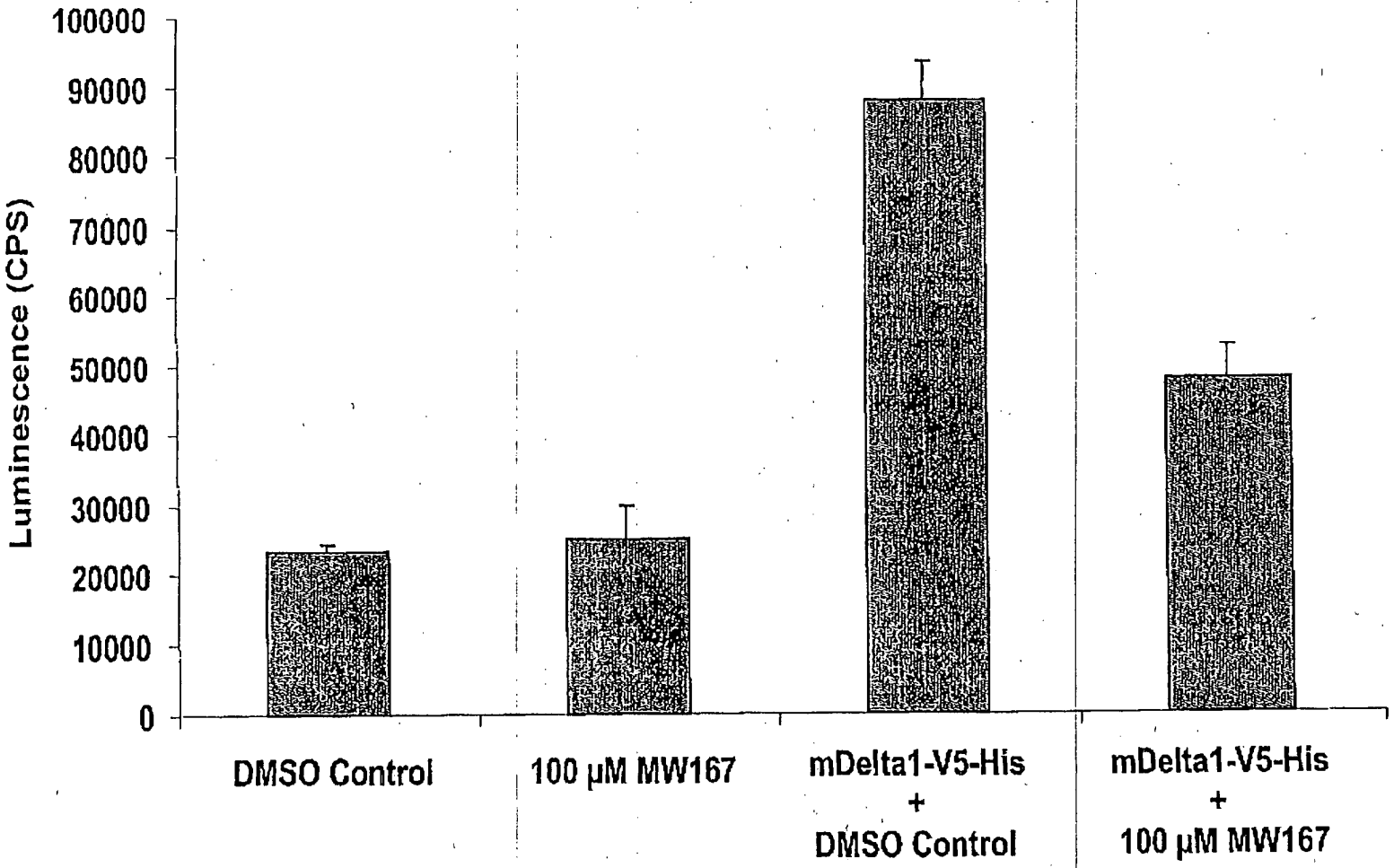


Figure 4